NATURAL RESOURCES AND THE ENVIRONMENT

Research in the area of natural resources and the environment addresses contemporary issues important for agriculture and for society. Knowledge in diverse scientific disciplines is needed in order to understand the influence of environmental fluctuations, to foster sustainability and economic viability, and to enhance stewardship of natural resources and agriculturally important ecosystems. Program Areas in this Division include: Plant Responses to the Environment; Forest/Rangeland/Crop/Aquatic Ecosystems; Soils and Soil Biology; and Water Resources Assessment and Protection. The Forest/Rangeland/Crop/Aquatic Ecosystems Program was not competed in FY 1998 due to limited funds. The Natural Resources and Environment Division also provides funding for the Improved Utilization of Wood and Wood Fiber Program; abstracts for this program can be found in the Enhancing Value and Use of Agricultural and Forest Products Division section.

PLANT RESPONSES TO THE ENVIRONMENT

Panel Manager - Dr. Theodore C. Hsiao, University of California, Davis
Program Director - Dr. Anne H. Datko

Awards in this area support research aimed at understanding the plant's response to environmental factors, both natural and anthropogenic. The major goal of the program is to provide the basic knowledge needed for devising strategies for decreasing the impact of environmental stress and for adapting agricultural and forest practices to possible changes predicted to accompany global climate fluctuations.

Studies on mechanisms at the whole plant, cellular or molecular level which explain organismal response are emphasized. The environmental factors of interest include water, temperature, light (including UV-B), nutrient, and atmospheric chemical composition (including carbon dioxide and other greenhouse gases, sulfur dioxide and ozone).

9800895 Photosystem-II Damage and Repair Cycle in Chloroplasts

Melis, A.
University of California, Berkeley; Department of Plant and Microbial Biology; Berkeley, CA 94720-3102
Grant 98-35100-6148; $210,000; 3 Years

This research investigates the repair of the photosynthetic apparatus from photo-oxidative protein damage, a common occurrence resulting from the abundance of oxygen and the presence of light energy in the vicinity of the oxygen-evolving photosystem-II of chloroplasts. This detrimental phenomenon occurs in all plants and algae under both physiological and adverse environmental conditions, causing an irreversible inhibition of the photosystem-II reaction center protein's function and potentially stopping photosynthesis.

Nature devised a complex and specific repair mechanism, unique in biology, that restores the functional status of photosystem-II. The essence of the repair is the selective removal, degradation and replacement of this photosystem-II reaction center protein. The repair process is of fundamental importance to biology but it is also of practical importance to agriculture because it maintains the productivity of photosynthesis. The goal of the research, through biochemical and molecular genetic approaches, is to elucidate the photosystem-II repair process. Mutagenesis by transformation of photosynthetic cells with tagged DNA will be employed in order to generate and isolate repair mutants. The tagged DNA will help to localize, sequence and study the genes and the enzymes that are involved in the repair of photosystem-II in chloroplasts. Elucidation of the repair mechanism will permit genetic manipulation of chloroplasts in order to enhance the repair function and, thus, to alleviate losses in plant growth and productivity that occur due to this photoinhibition.

9800807 Galactinol synthase: a key enzyme in plant stress tolerance?

Bradford, K.J.
University of California, Davis; Department of Vegetable Crops; Davis, CA 95616-8631
Grant 98-35100-6082; $164,250; 3 Years

The ability of seeds to dehydrate, survive for extended periods in the dry state, then rehydrate and resume growth is fundamental to the annual establishment of our most important crop plants. Seeds accumulate sucrose during the final stages of maturation, which interacts with cellular membranes and proteins to stabilize their structures as water is lost. In addition to sucrose, seeds also accumulate more complex sugars called oligosaccharides, primarily the raffinose family oligosaccharides (RFOs), which have been implicated in the acquisition of tolerance to dehydration and low temperature stresses in plants. While contributing to stabilization of membranes and proteins, RFOs also enhance the formation at low water contents of a physical condition known as the "glassy state." The extremely high viscosity of the glassy state retards the rates of chemical reactions that can lead to seed deterioration. The synthesis of RFOs is dependent on the sequential addition of galactose to sucrose from a compound called galactinol. Galactinol is formed by the action of galactinol synthase (GS), the rate-limiting enzyme step in the biosynthetic pathway. A cDNA coding for GS was isolated from tomato seeds. This cDNA will be used to characterize GS expression during tomato seed development and germination. The effects of treatments that alter seed longevity on GS expression, RFO content, and glassy state formation and stability will be determined. Mutants of corn or genetically engineered tomato plants unable to synthesize RFOs will be developed to evaluate the importance of RFOs in tolerance of desiccation and other stresses.

9801010 The Function and Transport of Boron in Plants. Implications for Agriculture and Biology

Brown, P.H.; Dandekar, A.M.
University of California, Davis; Department of Pomology; Davis, CA 95616
Grant 98-35100-3140; $120,450; 2 Years

Boron (B) is an element essential for plant life. However, the function of B in plants and the mechanisms that determine its distribution throughout plants are still poorly understood. In this project we will investigate the factors that determine B mobility in plants, use a new model system for the determination of B function and use genetic engineering techniques for enhanced B mobility and reduced susceptibility to reproductive B stress. In the previous award we demonstrated that B is phloem-mobile in all species for which either sorbitol, mannitol, or dulcitol is an important transported sugar. This includes the agricultural species celery, carrot, onion, pomegranate, apple, pear, peach, almond, cherry, plum, prune, and apricot. Boron is immobile in all other species and, hence, they are more sensitive to B deficiency than those with B mobility. Since the mobility of B is the primary determining factor in the management of B nutrition, this finding is of considerable agricultural and fundamental significance. This research will also lead to a clearer understanding of the physiological role of B in higher plants and provide an approach to the development of cultivars resistant to reproductive B deficiency, which would be of great agronomic significance.

9801027 Gordon Conference on Cellular Basis of Adaptation to Salt and Water Stress in Plants

Bray, E.A.
University of California, Riverside; Department of Botany and Plant Sciences; Riverside, CA 92521
Grant 98-35100-5990; $6,000; 1 Year

The Gordon Conference on the Cellular Basis of Adaptation to Salt and Water Stress in Plants will be held August 16-21, 1998 in Oxford, U.K. at Queen's College. The Chair of the Conference is Andrew Smith (University of Oxford) and the Vice-Chair is Elizabeth Bray (University of California, Riverside). It is the third conference in this series. The first Conference (held in August 1994) was focused solely on salinity. The second Conference (held in August 1996) was broadened to include cellular aspects of water stress and succeeded in attracting a wider range of researchers, bringing new ideas to both salt- and water-stress research, and ultimately increasing the potential impact of this Conference series on agriculture. The third conference will continue this tradition. The specific goals of this conference are: (1) to emphasize the importance of cellular and molecular approaches to understanding plant adaptation to environmental stress; (2) to highlight the latest progress in cell-level research on salt and water stress, particularly in the rapidly developing areas of ion transport proteins, ion and water channels, osmoprotectant biosynthesis, analysis of stress-induced gene expression, and dissection of the pathways of signal transduction; (3) to consider how advances at the cellular and molecular levels improve our understanding of adaptive responses, which may be applied in breeding or genetic engineering of plants for enhanced tolerance to water and salt stress; and, (4) to bring together a mix of established and beginning researchers from North America, Latin America, Europe, Japan and Australia and to promote extensive formal and informal discussions on current research problems and future opportunities.

9800809 Heat Shock Response in Higher Plants

Gallie, D.R.
University of California, Riverside; Department of Biochemistry; Riverside, CA 92521-0129
Grant 98-35100-6150; $190,000; 3 Years

The longest standing goal of agriculture has been to manipulate plant species to possess traits that are desirable by mankind, e.g., greater yields, improved quality and alterations in taste. Understanding how plants respond to changes in their environment at the level of gene expression is essential to any informed molecular genetic approach towards crop improvement and, as such, constitutes one of the fundamental goals of Plant Responses to the Environment program. Of all stress responses, the heat-shock response is most highly conserved from bacteria through higher eukaryotes. This ubiquitous response of organisms to thermal stress underscores the evolutionary need to meet this environmental challenge. Why study the effect of heat shock on translation and mRNA stability in plants? The ability to effectively respond to heat shock currently limits the regions and seasons in which many crops can be grown. Moreover, thermal stress can reduce growth and grain filling in cereals. In addition to the basic knowledge that our studies provide in understanding how translation and mRNA turnover function in plants, our findings are also revealing a mechanistic understanding of how protein synthesis is affected by heat shock. A greater understanding of the impact of thermal stress on translation and mRNA will be vital in the future to engineer new crop varieties for growth in new regions or to meet the challenge of changing climates due to global warming. The aim of our current proposal continues to be the role the changes in the regulation of translation and mRNA stability play in the heat shock response with a long term aim to increase tolerance for growth at elevated temperatures. Providing greater heat tolerance to these crop species may be vital for US agriculture to maintain sustainability in future environments.

9800817 Combining Chilling Tolerance at Emergence with Heat Tolerance at Flowering

Hall, A. E.
University of California, Riverside; Department of Botany and Plant Sciences; Riverside, CA 92521
Grant 98-35100-6129; $120,450; 3 Years.

In subtropical zones, chilling temperatures in spring can reduce emergence of warm season annual crops such as cowpea and cotton, whereas high temperatures in summer can reduce fruit or grain production of the same plantings. Varieties with more consistent and higher yields could be developed if genes that enhance chilling tolerance at emergence could be incorporated into plants together with genes that enhance heat tolerance at flowering. Currently it is not known whether these different types of tolerances can be combined in the same plant. I will attempt to breed varieties with tolerance to a broad range of temperatures using conventional hybridization and selection and will use cowpea, because genetic lines are already available in this species with either chilling tolerance at emergence or heat tolerance at flowering. It is only necessary to breed plants that combine these two complex traits, if it can be done. Physiological mechanisms of chilling tolerance during emergence, heat tolerance during flowering, and their interactions will be investigated. These studies could lead to the development of selection criteria, such as electrolyte leakage tests of membrane stability, and improved breeding methods that can be used to efficiently incorporate chilling tolerance during emergence and heat tolerance during flowering into cowpea and other warm season annual crops. In addition, proteins that confer stress tolerance in cowpea will be sought since genes conferring the synthesis of these proteins could be transferred to other crop species by genetic engineering.

9801002 Intracellular pH regulation and improved plant survival under low oxygen stress

Roberts, J.K.M.
University of California, Riverside; Department of Biochemistry; Riverside, CA 92521
Grant 98-35100-6146; $126,000; 2 Years

When the growing root tip of intact corn seedlings is subjected to hypoxic stress (3% 0_2), within as little as 2 hours it rapidly develops improved tolerance of environments completely lacking oxygen (anoxia). Crop plants commonly encounter hostile environments of hypoxia and anoxia, for example following heavy rains, and these stresses often cause reduction in yield - even complete loss of the crop. Selective protein synthesis during acclimation to low oxygen stress helps to prevent cytoplasmic acidosis, and so permits survival. The proposed work will provide the basis for understanding the mechanisms of intracellular pH regulation, and so the basis of metabolic tolerance of low oxygen stress. We will determine the contribution of carbohydrate metabolism to intracellular pH regulation during low oxygen stress. We will also test the following hypotheses: (1) Improved intracellular pH regulation in acclimated root tips is due to hypoxia-induced changes in the activities of cytoplasmic and mitochondrial enzymes which catalyze consumption or generation of protons. (2) During acclimation, translation is responsible for critical changes in metabolic patterns, extractable enzyme activities and intracellular pH regulation. These determinations must be made and hypotheses must be tested, if we are to understand how gene expression controls metabolism and how fundamental biochemical processes can improve whole plant performance under low oxygen stress.

9800713 Studies on the Mechanism of Heavy Metal Tolerance in *Arabidopsis*

Taiz, L.
University of California, Santa Cruz; Biology Department; Santa Cruz, CA 95064
Grant 98-35100-6058; $120,450; 2 Years

It is widely recognized that heavy metal pollution due to human mining, manufacturing and municipal waste disposal practices poses an ever-growing threat to agriculture and human health. Since plants readily take up a variety of potentially toxic heavy metals, they serve as an entry point for toxic heavy metals into the food chain. At the same time, heavy metals, when present in excess, are also toxic to plants, reducing crop productivity. Nevertheless, various metal-tolerant plant species have evolved which can thrive in metal-contaminated soils. Our research is focused on copper, an important and widespread heavy metal pollutant. The overal1 goal of our research is to elucidate the mechanism(s) of copper tolerance using *Arabidopsis thaliana* as a model system for studying copper homeostasis in a nontolerant species and the copper tolerant strain of *Mimulus guttatus*, "Copperopolis," as a model system for studying the mechanism of copper tolerance. Because copper has been shown to cause damage to cellular membranes, it has been proposed that membrane synthesis and repair are important in copper tolerance. We propose to study the effects of copper on the lipid composition of *Arabidopsis* and *Mimulus*. In addition, we will test the hypothesis that the trichomes of *M. guttatus* accumulate and secrete copper ions from the leaf.

9801009 Molecular Analysis of Inorganic Nutrient Sensing in Plants

Kim, E. J.
University of California, San Diego; Department of Biology, La Jolla, CA 92093
Postdoctoral Fellowship; grant 98-35100-6175; $90,000; 2 Years

As non-motile organisms, plants have evolved to adapt to the given external soil environment. It is well-documented that plants respond to changing levels of nutrients in the soil through a number of physiological changes, most notably in the activity of their uptake. However, the signal transduction mechanism underlying these responses remains largely unknown. I have recently identified a novel family of high-affinity potassium transporter genes (AtKUPs) from *Arabidopsis thaliana*. A member of this family has been shown to be specifically induced by withdrawing potassium from the medium and, therefore, may account for the induction of high-affinity potassium uptake observed in classical studies on plants upon K+-starvation. Induction of transport activity following starvation has also been observed in other transport systems involving other inorganic macronutrients, such as phosphate and nitrate. To identify genetic components participating in the sensing of these various nutrients, experiments are proposed to isolate mutants of *Arabidopsis* that have deficiencies in sensing of the macronutrients potassium, phosphate and nitrate. This will be achieved by use of transgenic plants harboring the luciferase gene as a reporter of induction, and identifying plants which show aberrant expression in inducing and/or repressing conditions.

9800877 Purification and Structural Analysis of Native Cold Stress Proteins

Guy, C.L.
University of Florida; Department of Environmental Horticulture; Gainesville, FL 32611
Grant 98-35100-6147; $72,270; 1 Year

Much of United States' major crop growing regions are subject to varying degrees of risk caused by freezing temperatures. The economic losses to the aggregate of all crop commodities in the last ten years at the farm gate could be conservatively estimated to be in the billions of dollars range. The process whereby some plants gain greater freezing tolerance when they are exposed to temperatures in the range of 32° to 50°F is not well understood. A primary objective of this project is to develop a better understanding of the molecular-genetic mechanisms that control freezing stress tolerance. The focus of this project is to determine the physical structure of two stress proteins that have been linked with freezing stress tolerance in spinach. These two proteins are accumulated when the plants are most tolerant of freezing stress and they disappear when the plants lose freezing stress tolerance. Their precise function in spinach is unknown. If the physical structure of the two proteins can be determined, unique features of their structure may provide valuable clues as to how they contribute to spinach freezing stress. The two proteins will be purified from cold treated spinach tissue or obtained from a recombinant expression system. Analyses will be conducted using the purified proteins to determine their secondary and tertiary structure. Models will be made of the predicted structures in an effort to identify important features that could indicate the role the proteins play in stress tolerance.

9801006 Heat stable mutants of maize endosperm ADP-glucose pyrophosphorylase

Hannah, L.C.; Greene, T. W.
University of Florida; Department of Horticultural Sciences; Gainesville, FL 32611
Grant 98-35100-6267; $164,250; 3 Years.

Yield loss due to increased temperatures ranges from 7 to 35% in the major cereal grains. Investigations showed that reduction in seed weight is a major contributor to this heat-stress induced loss. Furthermore, previous studies have shown that seed development separate from the plant is also adversely affected by temperature. Hence physiological processes of the seed are important in temperature-dependent decrease in seed weight. Previous studies point to temperature lability of two important starch synthetic enzymes, ADPglucose pyrophosphorylase (AGP) and soluble starch synthase (SSS) and have suggested that the temperature-dependent reduction in one of these enzymes is the cause of stress-induced loss in seed weight.

Replacement of the heat labile AGP with a more heat stable version may negate the heat induced loss in seed weight. Previously, we determined the feasibility of this approach by the use of a bacterial AGP expression system combined with mutagenesis. We mutated the corn seed gene, shrunken2 (Sh2), one of the genes encoding AGP and isolated heat stable variants.

In this work, we will: (1) isolate additional variants in the large and small subunit of AGP, (2) characterize the mutants and (3) place the mutants in whole corn plants to study the physiological consequence of the mutants.

9800831 Engineering Choline and Glycine Betaine Synthesis to Enhance Stress Tolerance

Hanson, A.D.
University of Florida; Department of Horticultural Sciences; Gainesville, FL 32611
Grant 98-35100-6149; $200,000; 3 Years

Drought and salinity are and always will be major environmental constraints on U.S. agricultural productivity. Some but not all plants respond to these stresses by synthesizing and accumulating compounds known as osmoprotectants, which help to prevent and reverse stress damage. One of the most effective of these compounds is glycine betaine (GlyBet). This work seeks the basic knowledge necessary to metabolically engineer the ability to synthesize GlyBet in plants that lack it. This requires the substrate choline and two genes, choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH). We have cloned both genes from spinach and sugar beet. To begin the engineering process we have expressed spinach CMO in a model plant, tobacco, which lacks CMO activity but has significant BADH. These plants produce GlyBet, but only in small amounts, and we have determined that this is because both the CMO activity and the endogenous choline supply are limiting. Thus we aim to increase CMO activity through applied molecular biology and to understand choline biosynthesis and metabolism in tobacco. Preliminary data indicate that the bottleneck in the choline supply is located at the first step of the synthesis pathway. Therefore, we will clone the spinach gene responsible for this step, phosphoethanolamine N-methyltransferase (P-EAMT), and use it to engineer tobacco to produce more choline. These plants will then be crossed with plants that express the GlyBet biosynthesis genes, and the resulting progeny will be analyzed for GlyBet as well as for salt and drought tolerance.

9800889 Whole-Plant Water Transport Phenomena: A Reassessment Incorporating Direct Measurement of Xylem Pressure and Knowledge of Water Channels

Meinzer, F.C.; Goldstein, G.
Hawaii Agriculture Research Center; Aiea, HI 06701
Grant 98-35100-6872; $109,500; 2 Years

The availability of water is often the most important factor limiting crop production. As water moves from the roots to the leaves in its journey along the soil/plant/atmosphere continuum, plants have several means of regulating its flow. The goal of this project is to reassess specific aspects of control of water movement through plants. We will employ a recently developed technique, the xylem pressure probe, which directly measures pressure in individual cells in the specialized water-conducting tissue of plants (xylem). Previously, only indirect techniques have been available for estimating the amount of tension or negative pressure that develops as water moves through the plant. Documenting the magnitude of pressure in the xylem is important for understanding the nature of the forces involved in moving water from the soil to the leaves as plants undergo the transpirational water loss that is an unavoidable consequence of uptake of carbon dioxide during photosynthesis. We will also examine the role of recently discovered, specialized water channel proteins in plant cell membranes in regulating xylem pressure and resistance to water movement through the plant. The results will lead to improved understanding of how physiological adjustments in different parts of the plant are coordinated to regulate plant water balance over different time scales and under different environmental conditions. This knowledge is fundamental for efforts to improve crop performance under both favorable and stressful conditions.

9801074 On the significance of location, activity and control of plant nitrate reductase

Cheeseman, J. M.
University of Illinois, Urbana-Champaign; Department of Plant Biology; Urbana, IL 61801
Grant 98-35100-6128; $137,000; 3 Years

Nitrogen fertilizers are a major economic input to agriculture, and in non-managed or "natural" ecosystems, nitrogen is a major limitation on growth, productivity and the biodiversity that a system can contain. The inefficient absorption of N fertilizers by plants is costly and can lead to environmental pollution. Inefficient use of N within plants can lead to toxic nitrate accumulations in tissues consumed by animals and people. The objective of this project is to use techniques of molecular genetics to create plants with different abilities and strategies for metabolizing the nitrate form of N provided by fertilizers, and to use them to study how plants as whole organisms integrate their use of this important nutrient. For the project, our main tools will be *Arabidopsis thaliana*, because of the great deal that is known about its genome at the molecular level, and peas, because of their importance as a crop and the new availability of techniques to manipulate the species genetically. Using mutants deficient in the ability to metabolize nitrate and the gene whose failure or absence is responsible for the deficiency, we will re-engineer the plants to have different locations, activities and controls on N metabolism. The results will be used in studies spanning the life cycle of both species to elucidate the long term effects of the manipulations on the overall growth and seed output of the plants.

9800878 Physical and Biological Constraints on Embolism Repair in Woody Plants

Holbrook, N.M.
Harvard University; Department of Organismic and Evolutionary Biology; Cambridge, MA 02138
New Investigator Award; grant 98-35100-6081; $175,000; 3 Years

Maintenance of water transport from soil to leaves is essential for plants. The ability to transport water through stems, however, may be lost or severely reduced due to the formation of air emboli within the conducting xylem vessels. Our study investigates the extent to which woody plants are able to repair these breaks in their water column. Specifically, we will document diurnal patterns in hydraulic conductivity in forest trees and investigate how the capacity for embolism repair is affected by plant water stress and tissue developmental state. We will also explore the physiological mechanisms behind the ability to restore hydraulic conductivity by examining embolism repair in low-lignin transgenic plants. Overall, the importance of this study is a better understanding of how both physics and physiology contribute to the overall dynamic nature of the water conducting system of woody plants.

9800827 Mechanisms Regulating Nitrogen Storage in Woody Perennials

Coleman, G.D.; Zhu, B.
University of Maryland; Department of Natural Resource Sciences and Landscape Architecture; College Park, MD 20742
Grant 98-35100-6108; $120,450; 2 Years

Environmental factors such as changing daylength (photoperiod) and temperature regulate many physiological and developmental processes in plants, including woody perennial plants. In many tree species, leaf nitrogen is resorbed during the fall and stored as protein (termed bark storage protein) during the winter in the stems. This resorption and storage of nitrogen is thought to be an important mechanism that may contribute to overall nitrogen use efficiency in trees. Using poplar (*Populus*) as a model, we will continue to investigate the physiological and molecular mechanisms that regulate the resorption and storage of nitrogen. Specifically this research will investigate what regions of the poplar bark storage gene are involved in environmental regulation of gene expression by characterizing the DNA sequences that interact with DNA-binding proteins, determine if leaf senescence is a prerequisite for bark storage protein gene expression, and investigate how changes in photoperiod regulate gene expression. The results of this research will contribute to understanding the mechanisms that regulate nitrogen resorption and storage in trees. Understanding how environmental factors regulate basic physiological processes in trees is important in developing strategies for improving tree productivity and predicting what effects environmental changes may have on tree growth and production.

9800810 Identification and Localization of UV-B Screening Compounds in Trees

Sullivan, J. H.
University of Maryland; Department of Natural Resources Sciences and Landscape Architecture;
College Park, MD 20742
Grant 98-35100-6127; $120,450; 2 Years

Concern over possible depletion of stratospheric ozone and concurrent increases in ultraviolet-B radiation (UV-B from 290 to 315 nm) has led to a significant amount of research over the past 2 decades. A wide range of effects of exposure to UV-B have been reported including photomorphogenic (growth) responses and changes in productivity. However, the observed wide range of responses or "sensitivity" to UV-B radiation makes it extremely difficult to predict how a given species will respond to a particular UV-B exposure level. The existing literature suggests that some of the variability in the response to UV-B radiation is due to differences in UV-protective mechanisms between species. One method of UV-protection that exhibits both adaptive and acclimation characteristics over the plant kingdom is the accumulation of secondary metabolites that absorb UV-B radiation and protect sensitive "target" molecules from damage by UV-B.

The degree of UV-protection afforded by screening compounds varies according to the molar absorptivity, quantities present and the localization of those compounds within the leaves. Unfortunately, very little information exists on the identification or localization of specific UV-screening compounds. The objective of this study is to test the hypothesis that UV-screening in tree species varies both quantitatively and qualitatively as well as by region of tissue localization. The effect of these differences in phenolics in altering the quantity and spectral quality of UV-B reaching the mesophyll will also be evaluated.

The results of this study will add to our knowledge of UV photobiology for trees and by extension, to other plant species, including crops. Since a direct photobiological response is dependent upon photon absorption by a receptor molecule, our understanding of the molecular, biochemical or physiological response to UV-B radiation will be limited until we understand the role of screening compounds in altering the internal UV-B environment in leaves. This research will benefit other ongoing research in this field such as the development of action spectra or in quantifying DNA damage or repair rates or other dose-response investigations.

9800836 Mechanisms of Selective Translation

Vayda, M.E.
University of Maine; Department of Biochemistry, Microbiology and Molecular Biology;
Orono, ME 04469-5735
Grant 98-35100-6034; $164,250; 3 Years

Mechanical wounding and low oxygen stress trigger both quantitative and qualitative changes in protein synthesis of potato tubers. Wounding causes mRNAs encoding tuber storage proteins to dissociate from poly-ribosome complexes at the same time as wound-response mRNAs accumulate and associate with ribosomes to be translated. Conversely, low oxygen stress causes a near-immediate arrest of protein synthesis which persists for several hours after onset of the stress. Wound-response mRNAs remain present during low oxygen stress but are not translated, even after translation has resumed. The objective of the proposed work is to understand the mechanisms which enhance protein synthesis in response to wounding, arrest protein synthesis at the onset of low oxygen stress, and select specific mRNA species for translation under both stress conditions.

The two specific objectives of this work will examine how protein synthesis is enhanced by association of ribosomes with the cytoskeleton, and will identify regions of mRNA sequences which mediate selective translation or association with cytoskeletal elements. We will compare the size and translational competence of free poly-ribosome complexes with poly-ribosome complexes in association with microfilaments. We will use radiolabeled mRNA segments synthesized *in vitro* in RNA/protein binding assays to identify protein factors and defined RNA sequences which may mediate selective translation.

Completion of these objectives will provide a detailed mechanistic understanding of how protein synthesis is regulated in response to stress conditions which affect the ability of plant tissues to resist opportunistic pathogens, affect crop quality and likely affect expression of transgene constructs under stress conditions.

9800879 Phosphorus-deficiency Induced Changes in Root Gene Expression of White Lupin

Allan, D.L.; Vance, C.P.
University of Minnesota, St. Paul; Department of Soil, Water, and Climate; St. Paul, MN 55108
Grant 98-35100-6098; $163,200; 3 Years.

An understanding of the mechanisms of nutrient acquisition by plants can help to amend nutrient deficiencies and reduce unneeded fertilizer inputs in agriculture. Among the major essential nutrients, our knowledge about uptake of soil phosphorus (P) is especially meager. The limitation of P deficiency to agricultural production is an enormous problem; more than 25% of the world's arable lands are limited in productivity due to P immobilization in the soil, and world phosphate rock reserves used to manufacture P fertilizers will last only 60-90 years if projected annual increases are correct.

Many plants adapted to low P conditions have long, fine roots or rely on mycorrhizal associations. White lupin is highly successful at obtaining P from low P soils by excretion of organic acids from proteoid roots. Proteoid roots are densely clustered, highly branched root zones which form in abundance when P is deficient. Our past research has characterized the development of proteoid roots and shown that P deficiency induces changes in their carbon metabolism which facilitate organic acid excretion. Concurrent secretion of acid phosphatase from proteoid roots is also an adaptive response to P deficiency. The goal of this project is to identify and characterize the genes that are involved in proteoid root development and the accompanying metabolic changes in the plant. We will isolate and characterize these genes, and then evaluate where and when they are expressed during the development of proteoid roots.

9800885 Interactions of Hsp30 with Other Proteins in the Heat Shock (Stress) Response

Plesofsky, N.S.; Brambl, R.M.
University of Minnesota, St. Paul; Department of Plant Biology; St. Paul, MN 55118-1095
Grant 98-35100-6038; $120,450; 2 Years

When exposed to moderately high temperatures, plants synthesize specific proteins that protect them from lethally high temperatures. These induced heat shock proteins include a prominent class of small proteins related to alpha-crystallin of the eye lens. How these small heat shock proteins contribute to thermotolerance has not been determined. Plants have several classes of these proteins encoded by multigene families, which makes it difficult to analyze the function of these proteins by gene disruption. The fungus *Neurospora crassa*, however, has only one gene for a small heat shock protein, Hsp30, which is abundantly produced at high temperature. To understand its role in thermotolerance, we blocked the synthesis of Hsp30 in *N. crassa* by disrupting its gene. We found that Hsp30 deficiency made fungal cells very sensitive to high temperature, when they were also nutritionally deprived of carbohydrate. Cells that lack Hsp30 show reduced ability to phosphorylate glucose at high temperature, a necessary step for glucose metabolism, and reduced import of proteins into mitochondria, the intracellular organelles in which carbohydrate is metabolized to yield energy. We will characterize the interaction of Hsp30 with proteins involved in glucose metabolism and energy generation. We will also determine which step(s) of protein import into mitochondria is destabilized at high temperature without Hsp30. These studies will increase our understanding of the specific physiological effects of high temperature stress on organisms and the contribution of a prominent heat shock protein in helping organisms maintain essential metabolic processes during this stress.

9800892 Molecular and Physiological Analysis of Heavy Metal Transport in a Unique Hyperaccumulating Plant Species

Kochian, L.V.
USDA Agricultural Research Service; U.S. Plant, Soil, and Nutrition Laboratory; Cornell University; Ithaca, NY 14853
Grant 98-35100-6105; $115,000; 2 Years

Recently, there has been a tremendous amount of interest in the use of terrestrial plants as a “green technology” for the phytoremediation of soils contaminated with toxic metals. A major factor behind the recent interest in phytoremediation of metal polluted soils has been the growing awareness by the scientific community of the existence of a number of metal hyperaccumulating plant species. We have been studying the best known of these hyperaccumulators, *Thlaspi caerulescens*, which is a zinc/cadmium hyperaccumulator. The unique physiology of heavy metal transport in *T. caerulescens* make it an interesting system for research aimed at elucidating mechanisms of heavy metal hyperaccumulation. The research to be undertaken with this funding will integrate molecular, biochemical and physiological approaches to identify and characterize mechanisms of heavy metal (Zn and Cd) hyperaccumulation in *Thlaspi caerulescens* in comparison with a related nonaccumulator, *Thlaspi arvense*. The studies will include physiological/biochemical investigations using radiotracer (^{65}Zn and ^{109}Cd) flux techniques to study compartmentation and sequestration in root and leaf tissues, as well as in tonoplast membrane vesicles isolated from roots and leaves. These findings will be integrated with molecular research focusing on characterization of a zinc transport gene we have recently cloned from *T. caerulescens*, along with several other clones that quite likely encode additional heavy metal transport genes in the hyperacumulator. The findings should enable researchers to better understand how plants remove toxic metals from the soil, and to introduce hyperaccumulation genes into agronomic plants in order to design plants that more effectively remediate contaminated soils.

9800829 Signal Transduction Events Leading to CAM Induction

Cushman, J.C.
Oklahoma State University; Department of Biochemistry and Molecular Biology; Stillwater, OK 74078-3035
Grant 98-35100-6035; $120,450; 2 Years

Soil salinization and competition for limited water resources in irrigated agriculture and drought in dryland agriculture are major limitations to crop productivity. Therefore, fundamental knowledge about stress-adaptive strategies in salt-loving, drought-tolerant plants, such as the common ice plant, *Mesembryanthemum crystallinum*, has important implications for maintaining and improving future productivity despite increasing soil salinization and competition for water resources. We have isolated a drought- and salinity-inducible gene from *M. crystallinum* that encodes a calcium-dependent protein kinase. Alterations in intracellular calcium play a central role in signaling events following stress perception. Therefore, calcium-dependent protein kinases are thought to be important sensors of altered calcium concentrations that occur following salinity and drought stress. Such signaling events ultimately control the expression of genes involved in adaptive responses such as the accumulation of compatible solutes or Crassulacean acid metabolism (CAM), an alternative mode of photosynthetic carbon fixation that improves water use efficiency. The goals of this project are to understand how this stress-induced calcium-dependent protein kinase functions in converting environmental stress signals to adaptive responses by determining its cellular and subcellular location, testing its effect on stress-adaptive gene expression, and identifying other cellular proteins with which it interacts. Understanding the components of calcium signaling pathways that mediate environmental stress responses will be essential for future efforts to manipulate stress adaptations at the level of master regulatory genes and to improve strategies for engineering salinity and drought tolerant crops.

9800870 Financial Support for the 12th Annual Penn State Symposium in Plant Physiology

Pell, Eva J.; Lynch, Jonathan P.
Pennsylvania State University; Department of Plant Pathology; University Park, PA 16802
Grant 98-35100-5989; $7,000; 1 Year

In several fields of plant biology there is growing recognition that phosphorus is a key regulator of plant processes, from cellular signal transduction and gene regulation, through metabolism and morphogenesis, to ecosystem productivity and response to global change. The goal of this meeting is to bring together leading researchers working at diverse levels of organization to consider this topic in an interdisciplinary context. The symposium will bring together 22 outstanding scientists from around the world to provide critical overviews of issues, challenges, and opportunities in a range of topics in cell and molecular biology, physiology, and ecology, with supporting perspectives from microbiology, phycology, and geology. In addition to invited talks we anticipate approximately 50 contributed posters summarizing current research by scientists, postdoctoral researchers, and graduate students. The proceedings of the symposium will be published by the American Society of Plant Physiologists, thus, providing a comprehensive and readily available introduction to the broad themes and issues related to the roles of phosphorus in plant biology, as well as in-depth treatment of areas of active research and poster “mini-papers” summarizing the latest findings. In addition, the symposium will stimulate discussion and debate among researchers working at distinct levels of organization and from diverse disciplinary perspectives. A major contribution of this symposium will be the formation of new collaborations and renewal of existing associations among researchers to within the international research community.

9800825 Molecular and Genetic Analysis of the Humidity Response in Stomatal Guard Cells

Watson, M.B.
Pennsylvania State University; Biology Department; University Park, PA 16802
Postdoctoral Fellowship; grant 98-35100-6162; $80,800; 2 Years

The growth of many crop plants can be limited by the amount of water in the environment. This limitation is due to the physiology and anatomy of plants. The problem is that for a plant to undergo photosynthesis it must have carbon dioxide. This gas diffuses into the plant leaf through a pore called a stomate. A pair of guard cells surround each stomate and control the size of the opening. When the stomata are open carbon dioxide diffuses in, but water is also lost by transpiration. Guard cells in the leaves respond to an increase in transpiration rate by closing the stomata, thereby, limiting water loss. Theoretically, guard cells could sense the total transpiration from: the leaves, the stomatal pore, the leaf surface or a combination of these. These studies will try to identify components of humidity signaling. One approach will be to assess the role of the cuticle or outer surface of the leaf. The humidity response will be measured in *Arabidopsis* plants with altered epicuticular wax formation. If water loss from cells surrounding the stomata influences the humidity response then plants with altered wax biosynthesis should exhibit an altered response to changes in humidity. In addition, we will try to isolate *Arabidopsis* plants that have altered response to humidity. That is, we will screen for mutant plants that cannot close their stomata in response to increased transpiration demand. Information related to how plants respond to transpiration could be useful for increasing the drought tolerance of crop species.

9800872 Gordon Research Conference on Temperature Stress in Plants

Guy, C.L.
Gordon Research Center; University of Rhode Island; West Kingston, RI 02892
Grant 98-35100-5991; $6,000; 1 Year.

The 1999 Gordon Research Conference on Temperature Stress in Plants is scheduled for late January at the Colony Harbortown Hotel in Ventura, California. The Gordon Research Foundation, the sponsor of the conference, provides significant but only partial funding for the conference. This award will help to make possible one of the most important and timely forums in the area of plant environmental stress research. The support will be used to help partially defray the expenses of session chairs, speakers and early career scientists to attend the conference. The 1999 Conference program will be organized around disciplines or noteworthy research approaches. Three aspects of temperature stress will form the unifying themes of the conference, a focus on compatible solutes and their metabolism, application of recombinant approaches to understanding stress responses and an integration of cellular and molecular processes within a larger climatic or global context. Keynote presentations will open and close the conference and help to focus emphasis on the major themes of the conference. The two keynote presentations will set the stage to view cellular and molecular issues within the larger context of climate and the global nature of life processes. Along with sessions on "Model Systems ," "Environment, Developmental Processes and Stress, ""Signal Transduction, Stress Perception and Responses,""Transcriptional and Translational Control of Stress Responses," "Molecular Chaperones," and "Biochemistry, Metabolism and Physiology of Stress" they will equally highlight the fact that global matters have their origins at the molecular and cellular levels. Discussions concerning the application of increased understanding of the temperature stress responses of plants will be focused towards appropriate agricultural situations.

9800828 Osmostress Tolerance in Plants: Role of Group 1 LEA Proteins

Marcotte, Jr., W.R.
Clemson University; Department of Biological Sciences; Clemson, SC 29634-1903

Grant 98-35100-6104; $150,000; 3 Years

A major class of proteins expressed during the latter stages of embryo maturation in angiosperms is called late embryogenesis abundant or LEA. Group 1 LEAs are typified by the wheat Em protein and Em-like genes have been identified in cotton, rice, maize, sunflower, barley, radish, *Arabidopsis*, carrot and mung bean. These proteins are rich in glycine and amino acids with charged R-groups and accumulation in the embryo is correlated with acquisition of desiccation tolerance. While Group 1 LEA proteins are not normally found in vegetative or immature seed tissues, expression is induced in response to osmotic stress. Therefore, these proteins may function as water stress protectants in both embryonic and vegetative tissues. Although the structural characteristics imply that Group 1 LEAs are capable of binding (or possibly replacing) large amounts of water, biochemical support for a role in water stress tolerance has been lacking. The goal of this project is to assess the ability of a Group 1 LEA protein to contribute to water stress tolerance in a whole plant system. This will be accomplished by generating transgenic plants expressing the wheat Group 1 LEA protein Em, both constitutively and inducibly, and analyzing the physiological response of the plants to water stress. The results from these studies could provide an opportunity to enhance resource (water) management in crop applications and to better maintain a balance between crop yield and environmental soundness. In addition, a plant-based system will be important for later dissection of the mechanism of protection through structure-function studies.

9800617 Cell Death and Aerenchyma Formation in Roots

Drew, M.C.; Morgan, P.W.; Cobb, B.G.
Texas A&M University, Department of Horticultural Science; College Station, TX 77843-2133

Grant 98-35100-6080; $120,450; 2 Years

When land becomes flooded or waterlogged, roots are deprived of a ready source of 0_2 from the soil air, and fail to function adequately in uptake of water and mineral nutrients, to the detriment of growth and yield. However, newly developing roots of maize, as well as the roots of many other cereal crop species, are induced by O_2 shortage to form internal airspaces (aerenchyma) that improve transfer of O_2 to root tissues from the air. This structural change helps plants resist waterlogging and is well-developed in plants of wetland origin, such as rice. In maize, aerenchyma formation takes place by the selective death of cells in the root that are sensitive to the plant hormone, ethylene. The synthesis of this hormone is accelerated in roots by O_2 shortage.

The overall aim of the proposed work is to improve understanding of how ethylene signals cell death. The specific objectives of the research are: 1) To determine the steps involved between initial exposure to ethylene and the death of root cells (the ethylene signal transduction pathway). This will involve the use of antagonists/agonists of the putative signal transduction pathway with maize to identify the components and their likely sequence. We will also measure directly changes induced by ethylene in the activity or concentration of phospholipase C and D and IP_3 which we suspect are involved. Complementary work will use the model plant *Arabidopsis thaliana*, for which we already have several mutants with an altered response to signaling by ethylene. 2) To determine whether proteases, especially cysteine/aspartate proteases (caspases), are involved in the initiation of cell death. In animal cells, caspases have a crucial role in the early events leading to programmed cell death. We will examine changes in activity of proteases, including caspases, in the root tips of maize during the promotion of cell death by ethylene. We will also test the hypothesis that there is conservation of genes associated with programmed cell death between animal and plant cells, using transgenic *Arabidopsis* plants, already in our lab, with overexpression of genes that regulate cell death in animal cells. Information obtain in this work will point the way toward future genetic improvement of flood-sensitive crops.

981030 A Phosphate Starvation-Inducible Purple Acid Phosphatase from *Arabidopsis*

McKnight, T.D.; Griffing, L.R.
Texas A&M University; Department of Biology; College Station, TX 77843

Grant 98-35100-6124; $149,000; 3 Years

Our long term goal is to understand factors involved in controlling phosphate metabolism in plants. Phosphorous, in the form of phosphate, is a major mineral nutrient for all organisms, including crop plants. In many soils, however, phosphate is not available in sufficient quantities to allow maximal plant growth. When phosphate is limiting, plants increase production of extracellular phosphatases. One role of these secreted phosphatases is to liberate phosphate from organophosphates in the environment. We are analyzing a gene encoding a secreted phosphatase from the model plant *Arabidopsis thaliana*. This gene is rapidly induced upon phosphate starvation, and analysis of its regulation and expression should increase our understanding of how plants respond to this type of stress. We will continue to analyze the regulation of this gene by levels of exogenous phosphate. The effects of endogenous phosphate levels will be examined also by analyzing expression of this gene in pho1 and pho2 mutants that accumulate less or more phosphate, respectively. This phosphatase is expressed in roots, flowers, leaves and seeds of *Arabidopsis*, suggesting that this enzyme has a broad role in phosphate metabolism. Tissue specific localization of the enzyme within these organs may suggest additional functions for the phosphatase. We are also interested in how plants detect the lack of phosphate. We are addressing this question by searching for mutants of *Arabidopsis* that cannot express the phosphatase gene when phosphate is limiting. If we can understand how plants detect and respond to suboptimal phosphate concentrations, we may be able to devise ways to improve crop growth and yield.

9800998 The Tonoplast H- ATPase and Salinity: Isoforms, Molecular Regulation and Impact

Binzel, M.L.
Texas A&M University, Texas Agricultural Experiment Station, EI Paso; E1 Paso, TX 79927
Grant 98-35100-6125; $175,000; 3 Years

Improving the salt tolerance of crops is an important and necessary strategy to cope with the increasing salinization of agricultural lands and to ensure the sustainability of U.S. agriculture. The use of irrigation is widespread; while required for production on arid and semi-arid land, it is being used increasingly in a supplemental fashion in semi-humid regions as well. Irrigation leads to the accumulation of salts in the soil surface which can reduce yields even in the most tolerant crops. Without a more comprehensive understanding of the pathways, mechanisms and genes responsible for salt tolerance, it will continue to be difficult, if not impossible, to make widespread and significant advances in crop tolerance to salinity .

The proposed research will examine the role of an enzyme (V-ATPase) considered to have an essential role in the response of plants to salinity. This enzyme generates the energy source that celIs use to accumulate and partition sodium ions. The ability to effectively sequester sodium is likely to be an important component of salt tolerance since sodium is toxic to most metabolic processes. It can be envisioned that an enhanced capacity to accumulate and partition sodium would be advantageous under saline conditions. Experiments will be conducted to examine how salinity alters the expression of two different forms of the catalytic subunit of this enzyme, what role these two different forms play, and how altering the composition of the enzyme with one or the other form of this subunit changes the properties of the enzyme.

9801000 Xylem water transport through roots of grasses and shrubs

Pockman, William T.
University of Texas at Austin; Department of Botany; Austin, TX 78713-7640
Postdoctoral Fellowship; Grant 98-35100-6079; $90,000; 2 Years

This project addresses the physical limits to water transport in grasses and shrubs of American rangelands. All plants lose water by evaporation through leaf pores (stomata) that are open to capture carbon dioxide during photosynthesis. To support continued growth, these losses must be replaced by water pulled through conducting tissue (xylem) from the soil via the roots to the leaves, not unlike drinking water through a straw. If the suction driving water movement becomes too great, as occurs when evaporation from leaves is too rapid or the soil is too dry, the xylem can become non-functional. This condition (cavitation) results from air entering the xylem producing an embolism and interrupting water movement. Cavitation occurs under conditions that differ widely among plant species. These differences help explain the variation in plant distribution across habitats with different patterns of water availability. Despite their central importance to rangeland agriculture, little is known about the role of cavitation in grasses or newly established shrubs. The proposed research will use a comparative approach to determine the limits of water transport in cool- and warm-season grasses and juveniles of two shrub species (Juniper and Mesquite) that are important in rangelands throughout the Southwestern U.S. Each species' ability to maintain water transport by remaining within the limits of its vascular system or controlling the loss of xylem function will be evaluated. The results will improve our understanding of the factors that contribute to whole plant responses to water stress in these under-studied grasses and young shrubs.

9801007 Spectral Balance, Spectral Weighting Functions and the Ozone Reduction Problem

Caldwell, M.
Utah State University; Department of Rangeland Resources and the Ecology Center; Logan, UT 84322
Grant 98-35100-6107; $175,200; 3 Years

How stratospheric ozone reduction affects terrestrial vegetation depends on how plants react to ultraviolet radiation of different quality (designated as wavelength) and also on how other regions of the solar spectum (e.g., the visible portion of the solar spectrum) modify these responses. Plants exposed to appreciable sunlight intensity in the visible portion of the spectrum tend to exhibit less ultraviolet radiation damage. A limited region of the ultraviolet spectrum called the UV-B is responsible for most of the sunlight UV damage and this is also the part of the spectrum that is most influenced by a depleting ozone layer. In nature, the ratio of UV-B to visible radiation is not constant. Under cloudy conditions or in shade, the visible portion of the spectrum tends to be decreased relative to the UV-B radiation. We will take measurements of light quality and consult existing data bases to generate "climatologies" of UV and visible radiation under these different conditions. Field experiments will be conducted using a special ultraviolet supplementation system together with various shading regimes to determine whether plant sensitivity increases or decreases as the proportions of light in the different spectral regions changes. Since USDA is the primary federal agency responsible for research on the effects of stratospheric ozone reduction on plants (both agricultural and nonagricultural), this work is highly relevant to agency objectives. The shading experiments will simulate realistic light regimes as experienced by weeds in crops, crops establishing under a protective cover crop, and many rangeland and forest species.

9801004 Temperature and Light Effect on Isoprene Basal Emission Rate

Sharkey, T.D.
University of Wisconsin, Madison; Department of Botany; Madison, WI 53706
Grant 98-35100-6036; $112,000; 2 Years

Isoprene emission helps some plants cope with short-term high temperature stress but can also contribute to ozone formation in polluted atmospheres. This research is a study of the effects of weather, both the amount of sunshine and temperature, on the ability of plants to emit isoprene. Oak trees, a model system for isoprene research, will be grown in highly controlled greenhouses with and without shade and at two different temperatures. Measurements of isoprene emission, the isoprene precursor, and other heat tolerance mechanisms will be made. Then trees will be moved from one environment to another and the rate of acclimation will be determined. This research will help predict the amount of isoprene being emitted as the weather changes allowing better predictions of crop-damaging ozone episodes and will provide insight into isoprene emission and other heat tolerance mechanisms. Improving our understanding of the various ways that high temperature affects photosynthesis and plant growth will allow the formulation of new strategies for improving heat tolerance of crops.

9800994 Biochemical gradients across Leaves: Influence of Light and Growth Conditions on High Light Tolerance

Nishio, J.N.; Vogelmann, T.C.
University of Wyoming; Department of Botany; Laramie, WY 82071-3165
Grant 98-35100-6106; $164,250; 3 Years

We are interested in how the spatial arrangement of metabolic activity within a leaf is related to how plants cope with environmental stress such as extremes in temperature or water availability. Environmental stresses in combination with natural levels of light can damage the photosynthetic apparatus. Such light damage, which often results in decreased crop productivity, is referred to as photoinhibition. How components of the photosynthetic apparatus and the induction of enzymes that can protect plants from photoinhibition relate to leaf productivity will be examined. The biochemical differences within leaves (from the top to the bottom) that are either tolerant or intolerant to excess light stress will then be related to leaf anatomy and the light environment measured within the leaves.

Our research follows a continuum from the whole plant to the molecular level. Metabolic parameters associated with photoinhibition will be measured in whole leaves, specific cell layers, and in the green membranes in chloroplasts. The changes in protein synthesis and enzyme activity associated with the parameters will also be examined under different environmental conditions.

Examining the relationship between leaf anatomy and photoinhibition may identify biochemical and anatomical traits that afford resistance to photoinhibitory damage in the field. Understanding how plants cope with environmental stress should lead to molecular and classical breeding solutions for improving plant productivity under less than optimal growing conditions that exist for most all agricultural situations around the world.

SOILS AND SOIL BIOLOGY

Panel Manager - Dr. Philippe Baveye, Cornell University
Program Director - Dr. R. Kelman Wieder

The Soils and Soil Biology Program supports research that will further our understanding of the basic mechanisms contributing to the immense diversity in soil chemical, physical and biological characteristics in both managed and unmanaged soils and sediments. The program was developed in recognition that soils provide the interface between the biotic and abiotic components of terrestrial ecosystems. It is in the soil that many of the essentials for the production of biomass are obtained and here that nutrients from dead biomass are recycled into usable forms.

9800840 Dynamic Spatial Patterns of Root C Input and Microbial N Processes Controlling Grassland N Cycling

Firestone, M. K.; Lindow, S. C.
University of California; Department of Environmental Science, Policy, and Management; Berkeley, CA 94720
Grant 98-35107-6369; $300,000; 2 Years

Nitrogen is the nutrient that most often limits productivity in natural and agronomic ecosystems. Many of the central transfers and transformations of nitrogen result from interactions between plants and soil microorganisms in the soil immediately surrounding roots, known as the rhizosphere. These transformations involve a complex interaction of organic matter in the soil, the organic exudates from roots, and the community of N-cycling soil microbes that are affected by these exudates and are localized in very fine-scale patterns around roots. Existing techniques for measuring these N cycling processes cannot provide the fine scale spatial resolution necessary because they involve disruption of the rhizosphere matrix being studied and require 10s or 100s of grams of soil for analysis. We now have techniques that allow us to measure all the important N cycling transformations at the fine scale. We will study N cycling associated with five representative grassland species, including important forage grasses and a noxious invading grass species. We will use genetically engineered bacteria containing reporter gene systems as micro-sensors to detect the pattern by which exudates including sugars and amino acids are released into the soil. We will use miniaturized assays to measure the microbial N transformations of: 1) asymbiotic N fixation, 2) N mineralization, and 3) nitrification and denitrification. The results will improve our basic understanding of nitrogen cycling, specifically the spatial and temporal patterns of soil N availability to plants.

9800782 Predicting Trace-Metal Bioavailability From Soil Solution Speciation: Can It Be Done?

Parker, D. R.
University of California, Riverside; Department of Environmental Sciences; Riverside, CA 92521
Grant 98-35107-6436; $160,000; 2 Years

The U.S. must safely dispose of large quantities of trace-metal-enriched waste products from municipal, industrial, and agricultural sources, and much of this disposal will continue to occur on agricultural lands. It has proven quite difficult to accurately predict the plant uptake and toxicity of trace metals. Better predictive capabilities are needed to properly assess the risks associated with waste disposal. It is widely believed that the plant availability of trace metals depends on their chemical form in soil solution, although surprisingly few studies have been conducted that quantitatively demonstrate such a relationship. This research will continue our critical reexamination of the correspondence between metal chemistry in the soil solution and plant toxicity of copper, a metal of importance in some sewage sludges and an increasing number of animal manures. Our approach will be to evaluate these relationships using experimental systems of increasing complexity. We will start with short-term root growth studies using "synthetic" soil solutions prepared in the laboratory using known compounds such as citric acid. We will then employ soils to which known quantities of Cu (as both sludge and Cu-salts) have been added, and use both short-term (two-day) root growth and longer term (one month) plant growth to assess the biological availability of copper. The predictive capability of the soil solution chemistry will thus be evaluated under simulated field conditions. The results should lead to improved conceptual models of trace metal bioavailability to soil-grown plants.

9800781 Molecular Characterization of Herbicide Biodegradation Capacity of Microbial Communities in Freshwater Wetlands, Intertidal Zones, and Agricultural Soils Using Atrazine as a Model

Radosevich, M.; Robinson, J.; Tuovinen, O.
University of Delaware, Department of Plant and Soil Sciences, Newark, DE 19717
Grant 98-35107-6388; $270,000; 3 Years

Relatively little is known concerning the processes by which natural microbial communities adapt to degrade and detoxify anthropogenic chemicals. An understanding of these processes is critical for improving risk assessment models and developing efficient remediation strategies. At a molecular ecology level, the most thoroughly investigated pesticide is 2,4-D which is widely used and is known to be degraded quite rapidly in soils. The implications of the recent research are that the 2,4-D-degradation pathway may have evolved from various pre-existing pathways being modified and combined in response to environmental exposure. Atrazine differs from 2,4-D in that atrazine is more recalcitrant, genes known to be involved in its degradative pathway are less frequently located on plasmids, and it can serve as both a C and N source. Thus, several regulatory systems may be involved in the induction and expression of both catabolism and assimilatory C and N metabolism in the atrazine-degrading genotypes. This study will assess the link between atrazine biodegradation rates and the frequency of genes encoding the degradative pathway of atrazine in soil microbial communities with varying exposure histories. Transformation and mineralization rates of atrazine and enumeration of atrazine-degrading populations will be determined in samples from wetland and agricultural sites. These data will be related to gene frequencies and expression levels for the atrazine degradative pathway. The results of the study will lead to a better understanding of the genotypic and phenotypic diversity of atrazine degradative pathways in wetland sediments and agricultural soils.

9800938 The role of Mimosine in Bacteria-Plant Interactions in the *Leucaena* Rhizosphere

Borthakur, D.
University of Hawaii; Department of Plant Molecular Physiology; Honolulu, HI 96822
Strengthening Award; Grant 98-35107-6492; $130,000; 2 Years

The rhizosphere of plants is a highly complex and dynamic ecosystem due to the continuous supply of organic material from the plants as root exudates. Many rhizosphere bacteria derive their nutrients by utilizing compounds secreted by the plant in the root exudates. We found that mimosine, a toxic free amino acid, is present in the root exudates of *Leucaena* which is an important tree-legume for tropical agroforestry. We found that some *Rhizobium* strains that form nitrogen-fixing root nodules on *Leucaena* can utilize mimosine as a source of nutrients. Thus, mimosine which is toxic to most other bacteria provides a selective advantage to certain *Leucaena*-modulating *Rhizobium* strains. We have cloned and identified five *Rhizobium* genes involved in mimosine degradation, and our preliminary results indicate that there are additional genes required for mimosine degradation. The objectives of this study are to (i) identify and characterize additional genes for mimosine degradation (ii) determine if the ability to catabolize mimosine is common to most bacteria in the *Leucaena* rhizosphere, and (iii) monitor the population dynamics of mimosine-degrading *Rhizobium* in the *Leucaena* rhizosphere. *Leucaena-Rhizobium* interactions through mimosine can be a model system to study the role of certain compounds in the root exudates for selection of beneficial bacteria in the rhizosphere. This work aims to establish an easy criterion to identify competitive *Rhizobium* strains for *Leucaena* and thus will help to develop an environmentally sustainable technology for agriculture and agroforestry.

9800751 Characterization of Amidase Produced by Soil Bacteria in Response to Polyacrylamide

Watwood, M. E.
Idaho State University; Department of Biological Sciences; Pocatello, ID 83209
Strengthening Award; Grant 98-35107-6952; $69,678; 2 Years

Soil erosion is a serious environmental and economic problem facing the U.S. Polyacrylamide (PAM) is a high molecular weight ($MW \geq 10^7$) compound, which reduces furrow-associated erosion by over 90%. The compound has a linear configuration and contains nitrogen in amide groups. It appears that treatment of soil with PAM stimulates soil microorganisms to produce specific enzymes that are able to access this nitrogen as a source of nutrition.

This project is to purify and characterize an amidase enzyme, produced by soil bacteria, which can biotransform PAM. We have found that exposure to PAM induces bacterial amidase activity, which releases nitrogen from the polymer chain as an ammonium. This enzyme appears to biotransform other amides in addition to PAM, including some amide-containing agrichemicals.

We plan to isolate and purify the PAM-active amidase from several soil bacterial isolates that exhibit activity in culture. The purified enzyme preparations will be characterized with respect to which compounds can serve as substrates and undergo biotransformation, how rapidly the enzyme works, and how various environmental factors can impact activity. Comparisons will be made among enzyme preparations from different isolates to determine if the same enzyme is produced by all bacteria exhibiting activity or if multiple forms are produced. We will also assess activity of the purified preparation toward other amide containing agrichemicals.

The results of this study will be an important component in defining the microbiological fate of PAM in agricultural soils and the potential impact of this anti-erosion additive on the fate of co-applied agrichemicals.

9800744 Abiotic Reductive Reactions of Organic Compounds on Microbially Reduced Clays

Stucki, J. W.; Yan, L.; Larson, R. A.
University of Illinois; Department of Natural Resources and Environmental Sciences; Urbana, IL 61801
Grant 98-35107-6313; $200,000; 2 Years

The fate of many herbicides (and other agriculturally important chemicals such as insecticides and fumigants) in the soil environment is poorly understood. In this project, we will observe the chemical transformation and decomposition of several organic chlorinated compounds by clays that have been converted to a reduced state (typical of zones below the surface of the soil) by microorganisms. We expect that such minerals should be especially effective in reacting with these compounds, unlike other clays, such as those that exist near the surface of the soil in an oxidized state. The focus of the study will be on reduced (ferrous) iron, which previous studies have indicated is the predominant form present on clay surfaces in subsurface environments as a result of the metabolic activity of natural microorganisms capable of living without free oxygen. Ferrous iron is likely to be the key reactant responsible for the degradation of organochlorine compounds and other reducible materials. In this research, two types of microbially reduced clays will be investigated for their efficiency to reduce approximately 10 different chlorinated compounds, all of which are important agricultural chemicals or environmental pollutants. The rates of disappearance of the test chemicals and the byproducts formed in their reactions will be determined. The results of the study should aid in our understanding of chemical transformation processes not only in agricultural soils, but also in other environments such as flooded regions, permanent wetlands, freshwater sediments, and contaminated groundwaters.

9800777 Tillage Impacts on Depth Distribution and Storage Capacity of SOM

Wander, M. M.; Spomer, A. L.; Mulvaney, R. L.
University of Illinois; Department of Natural Resources and Environmental Sciences; Urbana, IL 61801
Grant 98-35107-6387; $345,000; 3 Years

Forecasting the impacts of tillage practice on soil organic matter (SOM) characteristics may be one of the most important things we can do to predict and manage the future productivity of our soils. In many fine-textured soils, use of no-till (NT) practices has increased SOM contents at the soil surface at the expense of SOM stored in the rooting zone. Rapid shifts in particulate organic matter (POM) distribution that have resulted from NT adoption reflect dramatic changes in young SOM. This is of concern because young SOM had a disproportionately large impact on soil biological and physical condition. We must determine if SOM stratification in some Midwest soils is associated with restricted root activity at depth or is merely the product of root preference for a superior rooting environment. Decreased C inputs at depth may decrease the C storage capacity of NT soils. We suspect SOM stratification patterns reflect the physical condition of the soil and theorize that young SOM will only increase where residues are concentrated and SOM storage capacity is unfilled. We will assess the influence of porosity, penetration resistance, and aggregation and soil type on tillage impacts on SOM. Carbon-13 stable isotope techniques will be used to assess the C input patterns of roots and the effect those patterns have on aggregate protection of young SOM.

9800764 Microbial Community Dynamics in the Rhizosphere of *Zea mays* L. and *Glycine max* L.: Effects of Rotation and Tillage

Brouder, S. M.; Nakatsu, C. H.; Doerge, R. W.
Purdue University; Agronomy Department; West Lafayette, IN 47907-1150
New Investigator Award; Grant 98-35107-6389; $250,000; 3 Years

In much of the mid-western U.S., corn and soybean based production systems predominate. Monoculture yield decline occurs when either of these crops is grown continuously without rotation of the field into another crop. At present, this phenomenon remains poorly understood, but it has been linked to activities of soil microorganisms. To manage such obstacles to productivity, we must first identify and characterize the microorganism community in the rhizosphere, the micro-layer of soil surrounding roots. Molecular genetics now offers a number of promising tools with which to begin unraveling the complex ecology of soil microorganisms. The objective of this project is to use innovative molecular techniques to "fingerprint" rhizosphere communities and quantitatively relate them to root and shoot development, nutrient status and yield of corn and soybean when both crops are grown without rotation and when these crops are rotated annually with each other. Concurrent field and controlled environment experiments will be conducted. Interactions among rhizosphere soil microorganisms and differing host species will be assessed by characterizing and comparing the unique DNA "fingerprints" of microorganism communities (PCR amplification of 16s rDNA resolved by denaturing gradient gel electrophoresis). Quantitatively relating "fingerprints" to root form and function and crop development will advance our understanding of agricultural productivity and optimize cropping system management. Ultimately, our approach has the potential to revolutionize the way we select crop species for a rotation. "Fingerprinting" for beneficial and undesirable profiles in the rhizosphere may permit rapid screening for viable new or alternative crops to diversify current agroecosystems.

9800767 Soil-Mediated Productivity Vectors in an Alleycropping Agroforestry System

Gillespie, A. R.; Jose, S.
Purdue University, West Lafayette; Department of Forestry and Natural Resources; West Lafayette, IN 47907
Grant 98-35107-6314; $150,000; 3 Years

Agroforestry, the planting of trees and/or shrubs with agronomic crops or pasture, has been proposed as an alternative system for agricultural production on sensitive lands in the U.S., increasing long-term agricultural productivity while enhancing the natural resource base. Despite intense interest in these food and fiber production systems, the knowledge-base for research and implementation is only just developing, particularly for temperate regions. Several positive and negative ecological interactions among tree and crop components have been postulated for these integrated systems, but the relative importance of these interactions is unknown. Our research in red oak and black walnut alleycropping systems (the planting of crops in alleys formed by tree rows) over 10 years has shown that crop yields decrease as trees age. However, our current research has indicated that crop yields are limited by soil-mediated processes, not by light limitations as previously thought. The goal of this project is to determine the relative impact of tree competition for water vs. nitrogen. Using existing crop models, we will quantify nitrogen and water uptake by corn under alleycropping conditions with and without tree competition. Additionally, the effects of black walnut allelopathy (the exudation of harmful plant chemicals) on crop yield must be determined. Results of competition modelling will be validated with ^{15}N fertilizer trials. Quantification of water and nutrient competition and possible allelopathic effects will allow site- and soil-specific optimization of alleycropping system design to minimize negative interactions and yield loss. This will also allow the reduction of off-farm inputs and increase returns to the farmer for a system that already provides greater returns than either forestry or agricultural production alone.

9800796 Coupled Heat and Water Transport in Soils: A New Theoretical Development and Field Experimental Studies
Parlange, M. B.
Johns Hopkins University; Department of Geography and Environmental Engineering; Baltimore MD 21218
Grant 98-35107-6346; $163,000; 3 Years

The movement of water and heat in soils is basic to all soil science disciplines. Knowledge of heat and water movement is key to the growth of plants and transport of chemicals in the real agricultural environment. In particular, the coupled transport of heat and water needs to be understood for management of water and soil resources in the top one meter of the soil profile. This is the most valuable part of our soil resources. The current theoretical description of heat and water movement is based on diffusion theories. These theories are found to fail in the description of water vapor movement, which is the key to coupling water and heat movement in soils. We believe the problem with the current theory is that it does not account for the diurnal heating and warming of the soil air which leads to expansion and contraction and ultimately convective transport of the vapor. We plan to test measurements in the field with our new theory which is strongly supported with preliminary field data we have analyzed. This new theory will be critical in the description of the soil-plant-atmosphere continuum.

9800786 (Amino)Carboxylate Siderophores in Soils: Speciation, Surface Reactions, and Breakdown
Stone, A. T.
Johns Hopkins University; Department of Geography and Environmental Engineering; Baltimore, MD 21218
Grant 98-35107-6347; $156,000; 3 Years

(Amino)carboxylate siderophores released by the roots of grasses form chemical bonds to dissolved and mineral-bound ferric iron within soils and, in this way, facilitate iron acquisition. The efficacy of this iron uptake system affects crop success and depends on the siderophore employed and prevalent soil conditions. Large-volume crops, such as wheat, corn, barley, rice, and sorghum, use siderophores that differ in molecular structure and in the number and arrangement of carboxylic acid, amino, and alcohol groups. Protons, naturally-occurring metals (e.g. calcium), toxic metals (e.g. nickel and zinc), and mineral surfaces capable of adsorbing solutes (e.g. clays) compete with iron for available siderophores. Our objective is to develop a detailed understanding of (amino)carboxylate siderophore chemistry within soils. Capillary electrophoresis and other analytical methods are being developed to distinguish free-, iron-bound, and competitor metal-bound siderophores within soil fluids. Metal ion exchange, adsorption onto mineral surfaces, siderophore-assisted metal desorption, siderophore-assisted mineral dissolution, and siderophore chemical degradation are being investigated. The work is relevant to plant physiology and crop production, assessment and remediation of contaminated soils, and green chemistry applications. This work will be performed in collaboration with Dr. Rufus Chaney (USDA-ARS, Environmental Chemistry Laboratory).

9800798 Effect of Long-term Tillage and Cover Crop Systems on Soil Organic Matter and Pesticide Sorption
Xing, B.
University of Massachusetts, Amherst; Department of Plant and Soil Sciences; Stockbridge Hall, Amherst, MA 01003
New Investigator Award; Grant 98-35107-6319; $165,000; 3 Years

Soil organic matter (SOM) exercises a vital role in soil quality and sustainable agriculture. SOM is also the principal sorbent for pesticides and strongly regulates their behavior in soil. However, the effects of agricultural management on SOM changes, particularly the nature (e.g., structures, reactive groups, elemental compositions) and the relationships of these changes to pesticide sorption are poorly understood. Better knowledge on these issues is urgently needed for maintaining or improving soil and environmental quality. The goal of this research is to determine the SOM changes caused by agricultural practices, along with changes in pesticide sorption and mobility in soil using existing long-term tillage and cover crop plots. Spectroscopic methods involving advanced instruments will be used to elucidate SOM quality changes and its relationship with pesticide behavior. This study will lead to a mechanistic understanding of the effect of long-term tillage and cover crop systems on SOM quantity and quality. It will also advance our understanding of the relationship between SOM, pesticide chemistry in soil, soil quality, and agricultural management. Furthermore, the pesticide sorption study here is expected to provide results useful for assessing the efficacy and leaching of pesticides in soil and their availability to microorganisms, plants and animals. The results also will be useful for modifying existing pesticide leaching models to better predict the behavior of pesticides in soil. Overall, this proposed project will provide fundamental and instructive information for agricultural options to remain productive and competitive without having adverse environmental impacts.

9800839 Pesticide-Clay Interactions in Aqueous Systems
Boyd, S. A., Johnston, C. T.
Michigan State University; Department of Crop and Soil Sciences; East Lansing, MI 48824
Grant 98-35107-6348; $315,000; 3 Years

There are nearly 100 different herbicides currently used in the U.S. The total amount of herbicides applied annually in the U.S. exceeds 300 million pounds. In soils, organic matter and clays are potentially the two most active constituents responsible for the immobilization of soil-applied pesticides. Sorption of pesticides by these constituents plays a major role in determining their environmental fate, including pesticide leaching into groundwater, bioavailability to target pests, and biodegradation by soil microbes. Research over the past 15 years or more has focused on the singular role of soil organic matter as a sorptive phase for pesticides, while largely neglecting the role of soil clays. Clays were assumed to preferentially adsorb water, a process that rendered clay surfaces unavailable for pesticide adsorption. Most pesticide transport models now utilize soil organic matter normalized sorption coefficients to predict pesticide leaching in soils, an approach that ignores the contribution of pesticide sorption by clays. It is our contention that pesticide-clay interactions can be a major determinant of transport/retention processes in soils for several major classes of pesticides. In this project, we will systematically evaluate pesticide-clay interactions to determine classes of pesticides that are strongly immobilized by clays, and the molecular scale properties of clays and pesticides that influence these interactions. This knowledge is critical to improve risk management associated with pesticide use and to sustain the quality of our soil and water resources.

9800802 Soil Organic Matter Sorption of Hg and the Role of Reduced Organic Sulfur

Bloom, P. R.; Nater, E. A.
University of Minnesota; Department of Soil, Water, and Climate; St. Paul, MN 55108
Grant 98-35107-6315; $180,000; 3 Years

Elevated mercury in northern forest environments, caused by atmospheric deposition, is adversely impacting the utilization of fishery resources in national forests, and mercury added to agricultural land in waste products may also have adverse environment effects. The movement of mercury within soils and into streams and surface waters is strongly dependent on the bonding to natural organic matter in soils and in lake and stream water. The proposed project examines the strength and chemical structure of the bonding between natural organic matter and the mercury 2+ and methyl mercury 1+ ions. We hypothesize that the bonding is very strong and that it involves low oxidation state sulfur atoms in the organic matter. The strength of bonding will be determined by the equilibration studies using suspensions of soils, particulate aquatic organic matter, or extracted soil organic matter. Bromide will be added to the solutions to form soluble complex ions with mercury and yield easily measurable quantities of soluble mercury. Since the total mercury and bromide ion concentrations will be known, the quantity of soluble mercury that is not complexed can be calculated. The greater the ratio organic bonded mercury to non-complexed soluble mercury, the greater the strength of bonding. The chemical structure of the bonds will be determined using the x-ray spectroscopy capability of the National Synchrotron Light Source at the Brookhaven National Laboratory. The results will assist researchers in understanding the factors that control the mobility and biological transformations of mercury in the environment.

9800805 Structure and Dissemination of pADP-1, a Catabolic Plasmid Encoding Atrazine Degradation Ability

Sadowsky, M. J.; Wackett, L. P.
University of Minnesota; Department of Soil, Water, and Climate; St. Paul, MN 55108
Grant 98-35107-6368; $150,000; 2 Years

Atrazine is a widely used herbicide for the control of broad-leaf and grassy weeds in corn, sorghum and other crops. Because of its widespread use over the last 30 years, atrazine has been detected in soils and water in several countries. The use, environmental prevalence, and biodegradation of atrazine and related herbicides has assumed global significance in the last decade. We have developed and used a model system to examine bacterially-mediated atrazine biodegradation. We have previously isolated an atrazine-degrading bacterium, *Pseudomonas* strain ADP. The long-range goal of the proposed research is to provide a foundation for understanding the mechanisms involved in the assembly and dissemination of genes encoding atrazine degradation ability in bacteria. We have cloned, sequenced and expressed the first three genes involved in atrazine degradation in strain ADP and have shown that the atrazine degradation genes are localized on extrachromosomal (plasmid) DNA. This plasmid was named pADP-1. We have also investigated the distribution of these genes in other atrazine degrading bacteria on a global scale. In our studies we propose to characterize the novel atrazine degradation plasmid in *Pseudomonas* strain ADP and to determine whether similar plasmids are present in other bacterial strains that have the ability to degrade atrazine. Moreover, we propose to determine whether mobile genetic elements were involved in the assembly and evolution of pADP-1 and whether bacteria-to-bacteria gene transfer is one of the major mechanisms by which atrazine degradation ability has spread to geographically and genetically diverse soil bacteria.

9800789 Molecular Mechanisms of Bacterial Ferric Iron Mineral Reduction

Caccavo, Jr., F.
University of New Hampshire, Durham; Department of Microbiology; Durham, NH 03824
New Investigator/Strengthening Award; Grant 98-35107-6318; $210,000; 3 Years

Dissimilatory iron-reducing bacteria (DIRB) use Fe(III) as a sole terminal electron acceptor for anaerobic respiration. Although Fe(III) is abundant in the anaerobic soils and sediments in which many of these organisms typically are found, it is essentially insoluble and occurs as a variety of solid-phase oxides and oxyhydroxides. One of the major limitations to our understanding of the factors governing the rate and extent of dissimilatory Fe(III) reduction in soil and sedimentary environments is the unknown mechanism by which electrons are transported to insoluble Fe(III) oxides outside of the DIRB cell. I propose work that will begin to resolve this question. This work is a logical extension of my most recent studies, which suggest that the DIRB *Shewanella alga* produces a novel, cell-associated, iron-chelating protein (ICP) that can effectively solubilize Fe(III) from naturally-occurring iron oxyhydroxides. A comprehensive understanding of the significance of Fe(III) sequestration in DIRB now requires studies into: i) what role the ICP plays in Fe(III) oxide reduction; ii) where the ICP is located in the cell; and iii) how the cellular location of the ICP influences its function. This information will lead to a more complete understanding of the activity of Fe(III)-reducing bacteria in anaerobic environments and the biogeochemical cycle of iron. Such an understanding will favorably impact agricultural concerns, like the rice industry, that have been shown to be directly influenced by microbial Fe(III) reduction, and be valuable to efforts aimed at exploiting this metabolism in bioremediation.

9800773 Rhizosphere Processes and Soil Organic Matter Decomposition

Cheng, W.; Johnson, D. W.
Desert Research Institute, Biological Sciences Center, Reno, NV 89506
Grant 98-35107-6504; $345,000; 3 Years

Soil organic matter has long been recognized as one of the most important components in maintaining soil fertility, soil quality, and agricultural sustainability. The rhizosphere (the soil zone nearest the plant roots) plays an important role in regulating soil organic matter decomposition and nutrient cycling in agricultural systems. The linkage between rhizosphere processes and soil organic matter decomposition is not well understood. The goal of this project is to better explain the potential mechanisms controlling this linkage by addressing the following research questions: (1) Does soil mineral nutrition affect the direction and magnitude of root-soil interactions? (2) Do different plant species affect rhizosphere processes and soil organic matter decomposition differently? (3) Are there shifts in the magnitude of controlling mechanisms in the rhizosphere through time? In answering these questions, two major mechanisms will be examined: (1) mineral nutrient competition between plants and microorganisms living in the soil, and (2) stimulation or depression of microbial growth and metabolism caused by root activities. Completion of this study will improve understanding of rhizosphere processes and soil organic matter decomposition, thereby providing valuable information for better management and sustainability of agricultural systems.

9800754 Facilitated Transport of Metals in Sludge-Amended Soils

Steenhuis, T. S.; McBride, M. B.
Cornell University; Department of Agricultural and Biological Engineering; Ithaca, NY 14853
Grant 98-35107-6316; $165,000; 3 Years

Application of sewage sludges on agricultural lands is being widely promoted as a way of recycling the organic matter and nutrients present in sludges. However, sludges also contain trace (or "heavy") metals that can have adverse environmental effects. Conventional wisdom holds that metals in land-applied sludges are relatively immobile. However, when experimental systems or field sites are examined long after sludge application, a large fraction (often upwards of 50%) of sludge-applied trace metals is not accounted for in the soil. Researchers typically have concluded that this apparent loss is not due to leaching, based on no observed increases in metal concentrations at depth in the soil profile. However, the proposed alternative explanations of apparent losses, including lateral distribution by tillage or reversion to non-detectable mineral forms, appear to be inadequate. Preliminary investigations have shown substantial leaching of sludge-applied metals. Two factors appear to be enhancing metal mobility through the soil profile: 1) the presence of preferential flow paths (root channels, worm holes, structural cracks, etc. that allow water and solutes to rapidly bypass much of the soil matrix), and 2) metals complexing with soluble organics and/or colloidal materials that facilitate their transport through the soil profile. To investigate these factors we will continue to monitor the fate of sludge-borne metals in 90 undisturbed soil columns with a range of soil and sludge types. We will analyze the soil and leachate of these columns and that of an old application to better define the factors that facilitate metal (including Cd, Cr, Cu, Ni, Pb and Zn) transport from sludge-amended soils, and to determine which soil and sludge characteristics favor the formation of mobile forms. The results of this study will lead to a better understanding of the long-term environmental fate of sludge-borne metals and the potential risk of ground and surface water contamination.

9800739 Linking Biotic and Abiotic N-immobilization in Forest Soils

Dail, D. B.
Pennsylvania State University; Department of Agronomy; University Park, PA 16802
Postdoctoral Fellowship; Grant 98-35107-6317; $90,000; 2 Years

In many terrestrial environments, nitrogen (N) is the element most likely to limit growth of plants and soil organisms such as fungi and bacteria. Soil microorganisms are key to making nitrogen available to other animals, either through fixation of di-nitrogen gas (N_2), decomposition of dead plant material, or incorporation of nitrate (NO_3^-) that comes from acid rain. Industrial development and reliance on fossil fuels have increased the amount of N entering ecosystems in the form of nitrate. Nitrate may fuel productivity in soil microbes and increase growth in trees, however, excessive inputs can damage forest soils and drainage waters and even humans if ingested. One result of microbial use of nitrate is that much of the N gets tied up in soil organic matter (OM), a complex mixture of organic material derived from plant and microbial debris. The release of inorganic N from OM is slow when compared to other N sources and OM is, therefore, an important component of the resilience of forests to increased nitrate. Just how N becomes part of OM is the subject of much study, but it is probable that numerous pathways exist involving both biological and chemical reactions. It is my objective to investigate the relative importance of and links between biological and chemical N transformations. In particular, I will use new nuclear magnetic resonance (NMR) methods that I hope will indicate how N is transformed by soil microbes, in addition to the structure of soil organic N. This investigation may help us to understand the response of forest ecosystems to increased N deposition.

9800750 Enhanced C and N Mineralization in Earthworm Burrow Soil: Role of Ecological Interactions and Soil Pore Structure

Amador, J. A.; Görres, J. H.; Savin, M. C.

University of Rhode Island, Kingston; Department of Natural Resources Science; Kingston, RI 02881
Grant 98-35107-6320; $206,000; 2 Years

The activities of earthworms -- burrowing, fecal excretion and digestion -- enhance the availability of nutrients to crops in no-till agricultural ecosystems. How this enhancement occurs is not well understood. We hypothesize that, in the process of making burrows, earthworms engineer the soil to improve the habitat of soil microfauna, such as nematodes, feeding on bacteria and fungi. Specifically, we propose that compression of the soil by burrowing causes a shift to smaller soil pores that results in greater moisture retention, increasing the habitat accessible to nematodes. Enhanced nutrient cycling may result from increased feeding on bacteria and fungi by microbivorous nematodes. To test this hypothesis, we will measure: 1) the changes in soil structure (e.g. pore size distribution and pore volume) caused by burrowing, 2) the dynamics of bacteria and fungi and the nematode populations that graze on microorganisms in burrow soil, and 3) the production of inorganic nitrogen and carbon as a function of time in the burrows. Data will be obtained from earthworm addition experiments in the laboratory and in the field. This information will be used to develop a mechanistic model that describes the effects of earthworm burrows on nutrient cycling. This model will take into account changes in habitat, grazing of bacteria and fungi by nematodes, and weather-driven changes in soil moisture in order to predict nitrogen and carbon mineralization at the field scale. The results of this study should improve our ability to develop and manage sustainable agricultural practices that involve soil fauna.

9800838 Thickness of Organic Matter Coatings on Clay Mineral Surfaces

Rice, J. A.
South Dakota State University; Department of Chemistry & Biochemistry; Brookings, SD 57007-0896
Strengthening Award; Grant 98-35107-6515; $130,000; 2 Years

Humin represents the bulk of the organic carbon in soils and recent sediments. It is a major factor controlling contaminant fate and transport through the formation of what are known as +bound residues and represents a significant pool of organic carbon in the global carbon cycle. Its study is thus directly relevant to the long term goals of the sustainability of U.S. agriculture. Despite these compelling reasons, we know very little about humin’s physical or chemical nature. We do know that it is a composite of three organic matter fractions (bitumen, bound-humic acid, and bound-lipids) that represent distinct fraction of soil organic matter and a clay mineral component. This project will employ small-angel x-ray scattering and mathematical analysis of the scattering intensity data to calculate the thickness and volume of the adsorbed organic matter coatings on the clay mineral surfaces which comprise humin. It will make use of model systems (reference clay mineral adsorbents and surfactant and polymeric adsorbates) and authentic humin samples. The specific objective of this project is to calculate the thickness and volume of the organic coating on mineral surfaces. Ultimately, this research will examine the effect of environmental parameters such as pH, ionic strength and concentration of multivalent cations on the thickness of the coatings on mineral surfaces. The results of the study will provide, for the first time, a direct estimate of the amount (in the form of the thickness and volume) of the organic matter actually absorbed onto mineral surfaces. It is anticipated that the results of this study will provide the basis for further study of the architecture of humin, which can be used to study a variety of soil geochemical processes including bound residue formation and carbon cycling.

WATER RESOURCES ASSESSMENT AND PROTECTION

Panel Manager - Dr. Raymond E. Knighton, North Dakota State University
Program Director - Dr. R. Kelman Wieder

Land management and water use practices and policies affect water quality and availability, and habitat quality. Research is needed on the effects of farming, range, forestry, and other agricultural practices on our water resources, and to develop effective and economically feasible water pollution prevention or remediation practices.

Innovative research is supported on: (a) the distribution, fate, and transport of water-borne contaminants of agricultural origin; (b) the role of soil heterogeneity, hydrology, and landscape position on water quality; (c) management and remediation practices and/or technologies; (d) and the social, economic, policy, and environmental impacts of agricultural land management and water contamination remediation practices.

9800982 Cultivation of Halophytes to Reduce Agricultural Drainage Volumes.
California State University, Fresno; Department of Plant Science; Fresno, CA 93740-8033
New Investigator Award; Grant 98-35102-6531; $98,000; 2 Years

The disposal of agricultural drainage water has become a major problem facing growers in the westside San Joaquin Valley (SJV). In this region, subsurface drainage is critical to the management of high water tables and the protection of soils from salinization. Currently, drain water is stored in evaporation ponds or solar evaporators which are subject to EPA regulations on selenium and other trace elements in the water. These trace elements pose risks to waterfowl that visit the ponds. Drainage water reuse schemes, aimed at reducing drainage volumes through plant transpiration, have been proposed. The subsurface drain water would be re-utilized for irrigation in a sequential system which applies the drainage first to salt tolerant crops and then, after the drainage is even more concentrated, to halophytes (plants native to saline environments). We are studying salt tolerant plants and halophytes that could be included in drainage water reuse cropping systems. The plants must be tolerant of high levels of salinity and boron. It remains to be demonstrated that halophyte cultivation will substantially reduce drainage water volumes. The critical information needed is plant water use, especially for the halophytes, which are thought to have lower water use (evapotranspiration) rates than conventional crops. In our research, the growth and water use of four halophytes will be evaluated under irrigation with hypersaline, high boron, SJV drainage water. In the arid West, the use of saline water (drainage, surface or groundwater) for irrigation will become increasingly important as competition for freshwater supplies increases due to population pressure.

9800964 Assessment and Prediction of the Fate of Nitrate in Re-established Riparian Buffers
Isenhart, T. M.; Parkin, T. B.; Simpkins, W. G.; Schultz, R. C.
Iowa State University; Department of Forestry; Ames, IA 50011
Grant 98-35102-6810; $270,000; 3 Years

The goal of this work is to obtain a process level understanding of the mechanisms of nitrate transport and transformation in riparian (streamside) buffers re-established on previously cropped or pastured land. Specific objectives are 1) to utilize a groundwater and vadose zone monitoring network to define the spatial and temporal heterogeneity and evaluate mechanisms of nitrate attenuation in re-established riparian buffers, 2) to assess a chronosequence of re-established riparian buffer vegetation of 0 to 9 years of age for nitrate attenuation and compare these with riparian zones adjacent to crops, cool season grasses, and mixed timber, and 3) to develop a decision-aid for assessing the efficacy of re-established riparian buffers to attenuate nitrate. These goals will be achieved through intensive sampling and experimental approaches within the nearly 6.5 km of riparian buffer already established within the Bear Creek Basin in North Central Iowa and within an additional 6.5 km of buffer to be planted in 1998-1999. We will measure nitrate transport in shallow groundwater and vadose zone water and determine the relative importance of dilution, denitrification, or plant and microbial uptake in subsurface nitrate attenuation within these sites. This work will provide information to assist resource managers charged with developing and implementing restoration-based management approaches that build upon traditional water conservation and pollution control efforts. The information will also aid the success of the USDA National Conservation Buffer Initiative, a multi-year effort to encourage the use of landscape buffers to improve the environmental efficiency of farming.

9800858 Nitrate Removal from Subsurface Field Drains
Jaynes, D. B.; Kaspar, T. C.; Parkin, T. B.; Moorman, T. B.
USDA Agriculture Research Service; National Soil Tilth Laboratory; Ames, IA 50011
Grant 98-35102-6593; $100,000; 2 Years

Nitrate in water leaving field subsurface drain tiles often exceeds the maximum contaminant level set by EPA for drinking water and has been implicated in the hypoxia problem within the Gulf of Mexico. Because nitrate fertilizer management alone is not sufficient in reducing nitrate concentrations in tile drainage to acceptable levels, additional methods need to be devised. Enhancing denitrification (the transformation of nitrate by bacteria into harmless forms) around field tiles is a potential option for reducing nitrate concentrations. This research focuses on two methods for enhancing denitrification around field tiles. First, deep tile drain installation will be investigated for its potential to decrease nitrate concentrations in drainage waters. By installing tile drains approximately 3 feet deeper than currently practiced, while maintaining the drain outlets at current elevations, the submerged tile will be more efficient in drawing water from deeper saturated zones where nitrate concentrations are often much lower. Submergence may also encourage the formation of conditions around the tile that enhance denitrification. Second, we will examine the potential of adding wood-chip amendment to the soil surrounding tiles to form a denitrification biofilter barrier. Laboratory research will develop quantitative information on the effectiveness of wood-chips as a carbon source to support microbial denitrification. Wood-chip amended soil will also be added to soil surrounding tiles to measure effectiveness in the field. Both of these proposed approaches are passive in design, requiring no maintenance by the farmer after installation and are, thus, equally effective at any level of management.

9800966 Co-Migration of Metals and Dissolved Humic Substances in Aquifer Material

Thompson, M. L.; Hundal, L. S.; Carmo, A. M.
Iowa State University; Agronomy Department; Ames, IA 50011-1010
Grant 98-35102-6756; $252,000; 3 Years

This project is directed toward enhancing the long-term sustainability of U.S. agriculture by improving the assessment of risk to water resources associated with metal-contaminated soils. The research focuses on the role that humic substances play in promoting or retarding movement of toxic metals in groundwater. Soil and water quality criteria that are based on equilibrium partitioning of uncomplexed metal ions between solid and dissolved phases are inadequate predictors of the potential for metal migration. But there have been relatively few studies of the sorption and transport of humic complexes of metals in aquifer materials. Our preliminary data demonstrate that molecular size and hydrophobicity of humic substances are controlling factors in mobility of both dissolved humic compounds and complexed metals. The research will (1) evaluate the abilities of different kinds of dissolved humic substances to facilitate or retard sorption of copper and lead by aquifer materials, (2) correlate structural chemistry of humic fractions with their capacity to bind copper and lead, and (3) compare available metal transport models with a multiprocess, nonequilibrium model in predicting movement of organically complexed metals in aquifer material. We will use a sandy aquifer material from Iowa and humic components of sewage biosolids, fractionated by molecular weight and by hydrophobicity. Risk assessments of metal-contaminated soils and aquifers can be significantly improved if transport models recognize the role that organic complexation plays in mobility of transition-series and heavy metals.

9800919 Genetic markers for the identification of waterborne *Cryptosporidium parvum*.

Widmer, G; Tzipori S.
Tufts University School of Veterinary Medicine; Division of Infectious Diseases; North Grafton, MA 01536
Grant 98-35102-6757; $270,000; 3 Years

Waterborne *Cryptosporidium parvum* poses a significant threat to public health and is an important opportunistic infection in people with AIDS. Oocysts of *C. parvum* are commonly found in surface water. Calves are probably the main source of waterborne oocysts but the relative importance of human, agricultural and sylvatic sources for contamination of water with oocysts is unknown. Genetic markers for differentiating between oocysts from calves and from some human infections have recently been developed. The resolution of these markers is insufficient for the study of the transmission of this pathogen between animal and human sources. It is also unclear how stable these markers are when the parasite is transmitted between different hosts. Improved genetic fingerprints will facilitate the implementation of preventive measures aiming at reducing the number of waterborne oocysts by identifying environmental sources of oocysts. The long-term goal of this project is to develop an improved method for fingerprinting *C. parvum* oocysts found in surface water using the polymerase chain reaction. This will be achieved by identifying highly variable genetic sequences in the genome of the parasite. Subsequently, molecular methods for the detection and genetic fingerprinting of *C. parvum* in surface water will be optimized and the occurrence of *C. parvum* genotypes in local sources of surface water investigated. The stability of genetic fingerprints following transmission of *C. parvum* to animals will also be determined.

9800910 Tradable Permits for Controlling Nitrate Pollution of Domestic Groundwater Supplies

Eidman, V. R.; Coggins, J. S.; Nieber, J. L.
University of Minnesota; Department of Applied Economics; St. Paul, Mn 55108
Grant 98-35102-6528; $87,500; 2 Years

Nitrate contamination of municipal and domestic well water supplies is becoming a significant problem in many rural and urban areas, raising the cost of safe drinking water. This research uses production, soil, and groundwater models to address regional water quality problems in Olmsted County, Minnesota, where agricultural use of the land is intensive and nitrate contamination of groundwater is a growing concern. Crop rotation and fertilizer rates are determined by the production model. These two agricultural variables are incorporated into the soil model which predicts the subsequent nitrate leaching to groundwater. Given this quantity of leachate entering the aquifer, the groundwater model then approximates the resulting nitrate level at targeted wells. Integrating these three distinct models, we estimate the level of nitrates transported from farms in the county to targeted groundwater wells. Nitrate groundwater pollution has traditionally been analyzed as a nonpoint source problem. With our approach, farms can now be identified and held accountable for their specific portion of the contamination, thus converting the problem to a point source problem. Our research demonstrates how a tradable permit system can be used as a regulatory tool for controlling nitrate contamination of domestic groundwater supplies. Farms may engage in trades in this aquifer-based permit system as long as the nitrates delivered from their fields to the targeted groundwater wells do not exceed the nitrate standard set forth by the regional environmental authority.

9800859 Algal Community Responses to Ground and Surface Water Nutrient Exchanges in Agriculturally Dominated Settings
Warwick, J. J.; Vinyard, G. L.; McKay, W. A.
University of Nevada-Reno; Department of Environmental & Resource Sciences; Reno, NV 89512
Strengthening Award; Grant 98-35102-6578; $248,999; 3 Years

Much of the agricultural activity in the semi-arid western U. S. occurs adjacent to rivers and streams. In addition to serving the needs of the agricultural community, these waters are increasingly being looked upon to serve a multiplicity of other human needs (e.g. municipal and industrial water supply, and recreation) along with a variety of wildlife habitat needs. This growing diversity of demands is placing an ever increasing focus upon understanding the true dynamics of in-stream water quality and associated impacts on aquatic habitat. The Truckee River typifies rivers in the Great Basin by discharging to a terminal lake (Pyramid Lake), thereby focusing upstream water quality problems into a concentrating environment. Flood irrigation is the preferred method of water application in this region, thereby providing the requisite driving force to move waters and associated solutes through the vadose zone towards ultimate discharge into the river channel through the hyporheic zone. While considerable effort has been expended to date in the Truckee River Basin to identify point and nonpoint surface water inputs, groundwater inputs have been mostly ignored. Recent studies, utilizing numerical water quality models, support earlier work which suggests that there are significant subsurface inputs occurring in the Lower Truckee River.

The primary study goal is to conduct a fully integrated analysis of the impacts of agriculturally driven subsurface input on algal communities. We propose a multi-disciplinary methodology which incorporates nutrient flux field measurement, basic biologic analysis of periphyton response to differing nutrient flux regimes, and water quality simulation. This research will develop basic knowledge regarding the relationship between agricultural practices and aquatic ecosystem response. The experimental design will incorporate three, heavily instrumented, field sites experiencing both flood irrigation and no irrigation. We will specifically focus our attention on quantifying the role of the hyporheic zone as the point of nutrient storage and exchange with the in-channel flow. Bench scale algal chambers will allow for careful examination of the role of subsurface nutrient flux on the development of algal communities. Finally, these fundamental results will be integrated into a state-of-the-art water quality model to identify the potential significance of these findings at the watershed scale.

9800909 Distribution, Dissemination and Fate of *Cryptosporidium parvum* Oocysts
Bowman, D. D.; Jenkins, M. B.; Ghiorse, W. C.
Cornell University; Department of Microbiology and Immunology, Ithaca, NY 14853-6401
Grant 98-35102-6503; $210,000; 3 Years

Cryptosporidium parvum is a ubiquitous protozoan parasite of dairy calves around the world and a significant agent of waterborne gastroenteritis, e.g., in 1993, it caused a massive outbreak of gastroenteritis in over 400,000 of the citizens of Milwaukee, WI. Neonatal dairy calves are considered a major potential source of *C. parvum* for municipal watersheds. During a dairy calf's neonatal life, it can produce billions of infective oocysts that are suspected of contaminating surface waters and associated municipal watersheds. There is not, however, any data on how oocysts from these calves persist in the environment or move through soils into watersheds. We propose to determine rates of oocyst inactivation in soils with different biological activities at different temperatures and soil moisture contents. The distribution of oocysts around a known source of naturally infected calves will be determined in the field by intensive sampling of the soil around the calf hutches. Contour maps of oocyst frequency, viability, and environmental predictors will be generated and are expected to predict potential modes of oocyst dissemination. Dissemination scenarios will be tested by the application of fluorescent microbeads to the area followed by mapping of their movement. Sentinel oocysts will be used to identify rates of oocyst inactivation under field conditions. The results of this investigation will identify markers of potential oocyst inactivation in the field and ultimately allow farmers to define management practices to reduce the risk of agricultural runoff from polluting America's watersheds.

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9800906 Genetic and Engineering Improvement of a Swine Wastewater Plant Treatment System
Classen, J. J.; Cheng, J.; Bergmann, B. A.; Stomp, A. M.
University of North Carolina; NCSU; Biological & Agricultural Engineering; Raleigh, NC 27695
Grant 98-35102-6755; $300,000; 3 Years

We will develop a duckweed-based, remediation/production system that will recover nutrients from livestock wastewater in the form of valuable plant biomass. Plant-based, wastewater remediation systems have low installation and maintenance costs relative to conventional treatment plants. Two disadvantages are low nutrient recovery efficiency and no market for the biomass. We have developed a basic plant science and biological engineering collaboration to tackle these problems. Our strategy is to identify a small number of duckweed strains that are efficient for nutrient recovery and biomass production and determine what wastewater components limit growth and the tolerance of the selected strains to environmental perturbations. In parallel, we will test the selected strains in bench-scale remediation systems to determine nutrient recovery, duckweed growth, and harvesting performance as a function of system design. As we learn more basic information about what regulates nutrient recovery and growth, we will use this information in an iterative manner to match duckweed strains to the treatment system and the treatment system to the duckweed strains to optimize both wastewater remediation and duckweed biomass production. Gene transfer methods will also be developed for the selected duckweed strains to provide the tools to create new duckweed clones that make valuable products for which there is a market. With these tools, we will create a duckweed-based, wastewater treatment system that will transform livestock waste from an economic liability to a productive asset, will create the first, large-scale, aquatic crop plant, and will expand cropping systems to trillions of gallons of wastewater.

9800917 Lumped Parameter Models for Predicting Water Quality in Coastal Watersheds Skaggs, R. W.; Chescheir, G. M.; Gilliam, J. W.; Amatya, D. A.; Fernandez, G. P.
North Carolina State University; Dept of Bio & Ag Engineering; Raleigh, NC 27695-7625
Grant 98-35102-6493; $300,847; 3 Years

Water quality conditions leading to fish kills and health concerns in rivers and estuaries along the Atlantic and Gulf coasts are at least partially due to excessive nutrient loading. A large portion of these nutrients come from agricultural and silvicultural non-point sources. Reliable methods are available for estimating the effect of land uses and management practices on losses of nutrients and sediments at the field edge; however, methods to quantify these effects at the watershed scale are complex and nearly impossible to use for routine evaluations. The main product of this research will be lumped parameter models for predicting effects of land uses and management practices on nutrient and sediment loads from watersheds in the lower coastal plain. Development of lumped parameter models will include sensitivity analyses of existing complex water quality models to determine the relative importance of detailed parameters and which parameters can be lumped or neglected. Field measurements will be conducted to develop and test the models as well as provide independently useful data on processes affecting nutrient and sediment transport and fate in coastal watersheds. This field work will document probability distribution functions of water quality variables and the important parameters controlling those variables. Error analyses will be conducted on the lumped parameter models to determine the uncertainty of model predictions of monthly, seasonal and annual loads. The models developed in this project will facilitate watershed management decisions that reduce nutrient and sediment loading to receiving waters while maintaining or improving the productivity of coastal lands.

9800948 Point-Nonpoint Permit Trading Mechanisms to Reduce Costs and Increase Efficiency in Agricultural Pollution Control.
Randall, A.; Sohngen, B.
The Ohio State University; Department of Agricultural, Environmental, and Development Economics; Columbus, OH 43210
Grant 98-35102-6557 ; $130,000; 2 Years

In recent years there has been a noticeable movement from command-and-control pollution regulations in the United States toward incentive-based mechanisms, especially trading of pollution permits. While the program for trading sulfur dioxide permits is perhaps most noticeable, several point-nonpoint source pollution trading programs have been initiated in watersheds as well. Unfortunately, no trades have occurred in these programs, potentially due to their heavy focus on managing technology (i.e., BMPs) rather than performance (i.e., abatement).

We propose a comprehensive investigation into point-nonpoint permit trading in order to develop and refine trading mechanisms for controlling water pollution from agricultural sources. Objective (1): We will inventory, classify, and critically assess the diversity and magnitude of trading programs that have been implemented for the protection of water quality, wetlands, wildlife, and other land based resources. Objective (2): We will investigate the possibility that incentive-based pollution abatement programs can induce technological innovation in the industrial and agricultural sectors. Objective (3): We will investigate the prospects of developing effective performance-based point-nonpoint trading systems. This objective begins with theoretical static models, proceeds to dynamic simulation models, and explores the feasibility of linking the models to data from a watershed in Ohio. Objective (4): The findings of this research will be interpreted in a policy context, in order to expand and refine the array of permit trading institutions and mechanisms. Our findings will be published in both disciplinary and policy-oriented outlets, and will be communicated directly to policy-makers and stakeholders via extension channels.

9800958 Electrically Driven Microseparation Methods for Pesticides and Metabolites
El Rassi, Z.
Oklahoma State University; Department of Chemistry; Stillwater, OK 74078-3071
Grant 98-35102-6529; $132,000; 3 Years

The broad objective of our investigation entails the development of high performance capillary electrophoresis (HPCE) and capillary electrochromatography (CEC) methods for the detection of pesticides and their metabolites at low levels. This broad objective will be met by pursuing the following specific aims: (i) introduction of novel stationary phases for the rapid separation of pesticides and metabolites; (ii) development of laser-induced fluorescence (LIF) detection methods for the ultra sensitive determination of pesticides after HPCE and CEC separations; (iii) introduction of preconcentration schemes for dilute samples either by tandem CEC-HPCE or by on-line CEC in order to further enhance detectability; (iv) validation of the separation and detection methods with artificially polluted "real" waters. In objectives (i), the various stationary phases will be evaluated with widely differing pesticides under various conditions in order to determine the optimum separation conditions for maximum resolution. In objectives (ii) and (iii), the various LIF detection methods and trace enrichment approaches will be optimized and the figures of merit will be established. Finally, in objective (iv), the various separation and detection methods will be validated with "real" waters from various sources in order to assess the direct applicability of the proposed approaches to the analysis of pesticides and metabolites in "real world" samples. The various analytical methods described in this research project have the potentials of (1) increasing the accuracy of the analysis of pesticide and metabolite samples, (2) advancing the use of HPCE and CEC in monitoring processes relevant to water and (3) contributing to improving water quality.

9800975 Vitellogenin as a Biomarker for Estrogenic Chemicals in Watersheds

Selcer, K. W.
Duquesne University; Department of Biological Sciences; Pittsburgh, PA 15282
Strengthening Award; Grant 98-35102-6517; $130,000; 2 Years

A number of chemical pollutants have been found to mimic the physiological effects of the steroid hormone estrogen. Estrogenic chemicals include widely used pesticides and common by-products of the paper and plastics industries. Many chemicals from agricultural sources, such as herbicides and pesticides, are potentially estrogenic contaminants of the aquatic environment. An increasing body of evidence indicates that these estrogenic chemicals can have harmful effects on development and reproduction of wildlife and humans. Thus, it is important to be able to monitor environmental levels of these compounds. The various estrogenic pollutants cannot be detected using a single chemical assay; however, they can be measured by functional assays based on their similar biological activities. The objective of this proposal is to develop, test, and employ assays for evaluating the presence of biologically relevant concentrations of environmental estrogens in U. S. watersheds. These assays will use the serum protein vitellogenin as a biomarker of estrogenicity. Vitellogenin induction assays will be developed for several common aquatic vertebrates, including selected species of fish, frogs, and reptiles. These assays will be used in laboratory and field studies to assess the presence of estrogenic agents in various watersheds, including presumed pristine sites and known contaminated sites. The proposed research is relevant to the mission of the USDA in that it involves the development of a new method for detecting and measuring environmental contaminants, many of which are from agricultural sources.

9801079 Protecting Water Quality by Quantifying and Reducing Livestock Nutrient Excretion

Knowlton, K. F.
Virginia Polytechnic and State University; Department of Dairy Science; Blacksburg, VA 24061
Postdoctoral Fellowship; Grant 98-35102-6530; $90,000; 2 Years

The development of nutritional strategies to reduce nutrient excretion by livestock is an important and often overlooked opportunity to reduce nutrient loading to ground and surface water. The efficiency of nutrient conversion from feed to animal products is low, and most of the nitrogen and phosphorus consumed by livestock is excreted, but the variation observed indicates opportunity for improvement. This project will quantify and reduce potential nutrient pollution of ground and surface water by livestock in three ways. The first component of the project will develop management strategies to ameliorate the adverse effects of animal agriculture on water quality. I will develop and evaluate nutritional approaches to reduce nutrient excretion by dairy cows, testing different sources and levels of nutrient supplementation for their effect on nutrient excretion. The second component of the project will help predict quantity of waterborne contaminants. I will improve and evaluate a mathematical model (NutriMaP) which predicts nitrogen and phosphorus accumulation on dairy farms. The dairy herd is not explicitly included in current models which predict nutrient loading. I will develop and evaluate improved methods of predicting nutrient excretion by dairy cows and develop a unique method of predicting nutrient losses from manure storage. Finally the project will develop strategies to encourage acceptance of new technology to protect water quality. The NutriMaP model will be the cornerstone of an aggressive educational campaign to educate farmers and consultants on the potential of nutritional management to improve water quality while maintaining or improving profitability.

9800928 Managing Drip Irrigation to Reduce Chemical Movement in Soil

Felsot, A. S.; Evans, R. G.
Washington State University; Departments of Crop and Soil Sciences, Richland, WA 99352
Grant 98-35102-6516; $145,000; 2 Years

World wide interest in drip irrigation affords the opportunity for application of pesticides in a closed system with virtually no surface runoff, waste, or worker exposure. The subsequent distribution and leaching potential of drip chemigated pesticides, however, has been poorly studied. Before increasing numbers of growers adopt chemigation via drip irrigation systems, questions about leaching potential need to be answered, especially considering that the pesticides involved are comparatively high water solubility systemics. Two hypothesis are proposed to reduce the leaching potential of drip chemigated systemic pesticides. First, irrigating at a rate equal to crop evapotranspiration or a known matric potential matched to ideal crop growth will allay leaching by avoiding development of conditions conducive to saturated flow. Second, delaying watering after initial application will allay leaching by allowing greater time for equilibrium sorption to occur and increase intraparticulate diffusion to matrix spaces inaccessible to mobile water. These hypotheses will be tested using a combination of field and lab studies. Imidacloprid will be injected into a subsurface drip irrigation system installed in an experimental hop yard. Soil profiles will be collected from areas around water emitters in plots that are either irrigated on a daily 4-h schedule or irrigated when the matric potential falls to -0.155 bar. A superimposed treatment will be to start irrigation scheduling immediately after insecticide application or to delay irrigation for seven days. The overall goal of this project is to provide growers with a new pesticide application technology that is compatible with the need for environmental stewardship.

9800952 Oxygen Release from Plant Root Systems in Constructed Wetlands

Garbutt, K; Sexstone, A. J.
West Virginia University, Department of Biology, Morgantown, WV 26506

Grant 98-35102-6626; $159,986; 2 Years

Small-scale constructed wetland systems are promoted as an effective, low-cost method of improving effluent quality from both rural domestic wastewater and commercial agricultural waste streams. They may provide an alternative to centralized collection and treatment facilities, particularly in rural areas where traditional septic tank and drain fields fail due to steep terrain, impermeable geological strata, or shallow water tables. The treatment abilities of these systems has been inconsistent, however, and this research is designed to better understand the processes which facilitate treatment.

The release of oxygen by plant roots has long been hypothesized to be critical to many of these rooting-zone processes, including the microbial breakdown of organic matter. We will assay total oxygen release of entire root systems of numerous Appalachian wetland plant species, and also quantify finer-scale aspects of the release from particular regions of intact roots. Root systems will be digitally scanned and the image analyzed to obtain practical information on root architecture. Experiments will contrast release under full sun and dark conditions, allowing estimates of total daily oxygen release.

We expect species to vary in total oxygen release, in the proportions released by different portions of the root system, and in the proportion released into different regions of the rooting zone. Knowledge of these dynamics of oxygen release from a diversity of common wetland plant species will allow us to make practical recommendations of species and species mixtures for more detailed testing of their influence on chemical and microbial wastewater treatment processes.